RC
268.5
U55
no.49
1978

National Cancer Institute
CARCINOGENESIS
Technical Report Series
No. 49
1978

BIOASSAY OF ACRONYCINE FOR POSSIBLE CARCINOGENICITY

CAS No. 7008-42-6

NCI-CG-TR-49

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service
National Institutes of Health

Library
National Institutes of Health
Bethesda, Maryland 20014

United States. National Cancer Institute
Carcinogenesis technical report series

BIOASSAY OF
ACRONYCINE
FOR POSSIBLE CARCINOGENICITY

Carcinogenesis Testing Program
Division of Cancer Cause and Prevention
National Cancer Institute
National Institutes of Health
Bethesda, Maryland 20014

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service
National Institutes of Health

DHEW Publication No. (NIH) 78-849

RC
268.5
U55
no.49
1978

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE

National Institutes of Health

REPORT ON BIOASSAY OF ACRONYCINE FOR POSSIBLE CARCINOGENICITY

Availability

Acronycine (CAS 7008-42-6) has been tested for cancer-causing activity with rats and mice in the Carcinogenesis Testing Program, Division of Cancer Cause and Prevention, National Cancer Institute. A report is available to the public.

Summary: A bioassay of acronycine for possible carcinogenicity was conducted by administering the test chemical by intraperitoneal injection to Sprague-Dawley rats and B6C3F1 mice.

Initially, groups of 35 rats of each sex were administered acronycine at one of two doses, either 7.5 or 15 mg/kg body weight, in a vehicle composed of 0.05 percent polysorbate 80 in phosphate-buffered saline. Control groups of each sex consisted of 10 untreated rats (untreated controls) and 10 rats injected with the vehicle (vehicle controls). Because of high mortality rates in the dosed animals, new dosed groups of 35 rats of each sex were started later at a dose of 3.75 mg/kg. Additional groups of 10 untreated and 10 vehicle controls of each sex were also started. The rats were administered the acronycine or the vehicle for 51 or 52 weeks, then observed for an additional 28-30 weeks. All surviving rats were killed at 80-82 weeks.

RC
268.5
U55
no. 49
1978

Initially, groups of 35 mice of each sex were administered acronycine at one of two doses, either 12.5 or 25 mg/kg body weight, in a vehicle composed of 0.05 percent polysorbate 80 in phosphate-buffered saline. Control groups of each sex consisted of 10 untreated mice (untreated controls) and 10 mice injected with the vehicle (vehicle controls). Because of high mortality rates in the dosed animals, two additional dosed groups were started later: 35 mice of each sex at 6 mg/kg and 40 mice of each sex at 2 mg/kg, together with untreated controls and 10 vehicle controls of each sex for the groups dosed at 6 mg/kg, and 20 untreated controls and 20 vehicle controls for the groups dosed at 2 mg/kg. Periods of administration of the chemical to the mice varied from 25 weeks to 92 weeks, depending on toxicity or length of time of survival. Surviving control animals were killed at 78-105 weeks.

Acronycine was toxic to rats and mice of each sex at the doses used in this bioassay, as shown by the high mortality rates in all but the low-dose groups and by the lower mean body weights in dosed rats and mice at all doses throughout most of the bioassay. Because of this high number of deaths, time-adjusted statistics are used for the analyses of all incidences of tumors.

It is concluded that under the conditions of this bioassay, the low survival of the dosed and control mice and the possible procedural problems associated with the intraperitoneal injection of the chemical did not allow a determination to be made of the carcinogenicity of acronycine in this species. In Sprague-Dawley

RC
268.5
U55
no. 49
1978

rats, acronycine in the vehicle of 0.05 percent polysorbate 80 in phosphate-buffered saline was carcinogenic, producing tumors of the mammary gland in females, osteosarcomas in males, and sarcomas and other related tumors of the peritoneum in both males and females.

Single copies of the report are available from the Office of Cancer Communications, National Cancer Institute, Building 31, Room 10A21, National Institutes of Health, Bethesda, Maryland 20014.

Dated: July 11, 1978

Director
National Institutes of Health

(Catalogue of Federal Domestic Assistance Program Number 13.393, Cancer Cause and Prevention Research)

BIOASSAY OF
ACRONYCINE
FOR POSSIBLE CARCINOGENICITY

Carcinogenesis Testing Program
Division of Cancer Cause and Prevention
National Cancer Institute
National Institutes of Health

FOREWORD: This report presents the results of the bioassay of acronycine conducted for the Carcinogenesis Testing Program, Division of Cancer Cause and Prevention, National Cancer Institute (NCI), National Institutes of Health, Bethesda, Maryland. This is one of a series of experiments designed to determine whether selected chemicals have the capacity to produce cancer in animals. Negative results, in which the test animals do not have a greater incidence of cancer than control animals, do not necessarily mean that the test chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of circumstances. Positive results demonstrate that the test chemical is carcinogenic for animals under the conditions of the test and indicate that exposure to the chemical is a potential risk to man. The actual determination of the risk to man from animal carcinogens requires a wider analysis.

CONTRIBUTORS: This bioassay of acronycine was conducted by Southern Research Institute, Birmingham, Alabama, initially under direct contract to the NCI and currently under a subcontract to Tracor Jitco, Inc., prime contractor for the NCI Carcinogenesis Testing Program.

The experimental design and doses were determined by Drs. D. P. Griswold[1], J. D. Prejean[1], E. K. Weisburger[2], and J. H. Weisburger[2,3]. Ms. J. Belzer[1] and Mr. I. Brown[1] were responsible for the care of the laboratory animals and the administration of the test chemical. Data management and retrieval were performed by Ms. C. A. Dominick[1]. Histopathologic examinations were performed by Drs. S. D. Kosanke[1] and J. C. Peckham[1], and the diagnoses included in this report represent their interpretation. The reported neoplasms and chemical-related lesions were reviewed

by Dr. J. F. Hardisty[4], who prepared the interpretive pathology summary included in this report.

Animal pathology tables and survival tables were compiled at EG&G Mason Research Institute[5]. The statistical analyses were performed by Dr. J. R. Joiner[6], using methods selected for the bioassay program by Dr. J. J. Gart[7]. Chemicals used in this bioassay were obtained through Mr. C. A. Hewitt[9]. Chemical analyses were performed by Drs. J. Stewart[8] and R. H. Iwamoto[8], and the analytical results were reviewed by Dr. S. S. Olin[6]. The structural formula was supplied by NCI[2].

This report was prepared at Tracor Jitco[6] under the direction of NCI. Those responsible for the report at Tracor Jitco were Dr. Marshall Steinberg, Director of the Bioassay Program; Dr. L. A. Campbell, Deputy Director for Science; Drs. J. F. Robens and C. H. Williams, toxicologists; Dr. R. L. Schueler, pathologist; Dr. G. L. Miller, Ms. M. S. King and Mr. W. D. Reichardt, bioscience writers; and Dr. E. W. Gunberg, technical editor, assisted by Ms. Y. E. Presley.

The statistical analysis was reviewed by members of the Mathematical Statistics and Applied Mathematics Section of NCI[7]: Dr. John J. Gart, Mr. Jun-mo Nam, Dr. Hugh M. Pettigrew, and Dr. Robert E. Tarone.

The following other scientists at NCI[2] were responsible for evaluating the bioassay experiment, interpreting the results, and reporting the findings: Dr. Kenneth C. Chu, Dr. Cipriano Cueto, Jr., Dr. J. Fielding Douglas, Dr. Dawn G. Goodman, Dr. Richard A. Griesemer, Dr. Harry A. Milman, Dr. Thomas W. Orme, Dr. Robert A. Squire[10], and Dr. Jerrold M. Ward.

---

[1] Southern Research Institute, 2000 Ninth Avenue South, Birmingham, Alabama.

[2] Carcinogenesis Testing Program, Division of Cancer Cause and Prevention, National Cancer Institute, National Institutes of Health, Bethesda, Maryland.

[3] Now with the Naylor Dana Institute for Disease Prevention, American Health Foundation, Hammond House Road, Valhalla, New York.

[4] Experimental Pathology Laboratories, P.O. Box 474, Herndon, Virginia.

[5] EG&G Mason Research Institute, 1530 East Jefferson Street, Rockville, Maryland.

[6] Tracor Jitco, Inc., 1776 East Jefferson Street, Rockville, Maryland.

[7] Mathematical Statistics and Applied Mathematics Section, Biometry Branch, Field Studies and Statistics, Division of Cancer Cause and Prevention, National Cancer Institute, National Institutes of Health, Bethesda, Maryland.

[8] Stanford Research Institute, Life Sciences Division, Menlo Park, California.

[9] Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Bethesda, Maryland.

[10] Now with the Division of Comparative Medicine, Johns Hopkins University, School of Medicine, Traylor Building, Baltimore, Maryland.

SUMMARY

A bioassay of acronycine for possible carcinogenicity was conducted by administering the test chemical by intraperitoneal injection to Sprague-Dawley rats and B6C3F1 mice.

Initially, groups of 35 rats of each sex were administered acronycine at one of two doses, either 7.5 or 15 mg/kg body weight, in a vehicle composed of 0.05% polysorbate 80 in phosphate-buffered saline. Control groups of each sex consisted of 10 untreated rats (untreated controls) and 10 rats injected with the vehicle (vehicle controls). Because of high mortality rates in the dosed animals, new dosed groups of 35 rats of each sex were started later at a dose of 3.75 mg/kg. Additional groups of 10 untreated and 10 vehicle controls of each sex were also started. The rats were administered the acronycine or the vehicle for 51 or 52 weeks, then observed for an additional 28-30 weeks. All surviving rats were killed at 80-82 weeks.

Initially, groups of 35 mice of each sex were administered acronycine at one of two doses, either 12.5 or 25 mg/kg body weight, in a vehicle composed of 0.05% polysorbate 80 in phosphate-buffered saline. Control groups of each sex consisted of 10 untreated mice (untreated controls) and 10 mice injected with the vehicle (vehicle controls). Because of high mortality rates in the dosed animals, two additional dosed groups were started later: 35 mice of each sex at 6 mg/kg and 40 mice of each sex at 2 mg/kg, together with 10 untreated controls and 10 vehicle controls of each sex for the groups dosed at 6 mg/kg, and 20 untreated controls and 20 vehicle controls for the groups dosed at 2 mg/kg. Periods of administration of the chemical to the mice varied from 25 weeks to 92 weeks, depending on toxicity or length of time of survival. Surviving control animals were killed at 78-105 weeks.

Acronycine was toxic to rats and mice of each sex at the doses used in this bioassay, as shown by the high mortality rates in all but the low-dose groups and by the lower mean body weights in dosed rats and mice at all doses throughout most of the bioassay.

Because of this high number of deaths, time-adjusted statistics are used for the analyses of all incidences of tumors.

In male rats, the dose-related trend in the mid- and high-dose groups for the incidence of osteosarcoma at all sites was significant (P = 0.002) using the respective vehicle-control group (vehicle controls 0/8, mid-dose 13/30, high-dose 12/18). Comparisons of the individual groups with respective control groups were also significant for the mid-dose (P = 0.022) and high-dose (P = 0.002) groups, but not for the low-dose group. In female rats, osteosarcoma was observed only in 1/8 high-dose animals.

Sarcomas and other related tumors of the peritoneum were observed in all three dosed groups of both male and female rats, but in none of the control groups (males: low-dose 5/30, mid-dose 3/26, high-dose 7/16; females: low-dose 1/35, mid-dose 5/30, high-dose 13/28). In both sexes, the dose-related trends were significant (males, P = 0.006; females, P = 0.002), and the comparison of the incidences in the high-dose females with the vehicle-control group was significant (P = 0.016). None of the incidences in the individual dosed groups of males were significant when compared with vehicle controls. However, since the tumors were observed in all dosed groups but did not occur in historical-control animals at this laboratory, they are considered to be related to the administration of the chemical.

In female rats, the incidence of all tumors of epithelial origin of the mammary gland was significant only at the low dose (low-dose vehicle controls 1/10, low-dose 22/35, P = 0.004). Adenocarcinomas of the mammary gland were observed in seven low-dose, five mid-dose, and two high-dose female rats, but in no control females. The reverse dose relationship of both benign and malignant tumors was probably due to the higher number of early deaths which occurred in the high-dose group.

In mice, the low survival in all dosed groups except the low-dose animals precluded an evaluation of the significance of the incidences of tumors. Lymphomas occurred in low-dose groups of both males and females; however, the incidence of lymphoma in different control groups was highly variable. The high incidence in the low-dose vehicle controls may have been due to a procedural problem associated with the possibility of transfer of tumor cells or oncogenic viruses during the intraperitoneal injection of the test chemcial.

It is concluded that under the conditions of this bioassay, the low survival of the dosed and control mice and the possible procedural problems associated with the intraperitoneal injection of the chemical did not allow a determination to be made of the carcinogenicity of acronycine in this species. In Sprague-Dawley rats, acronycine in the vehicle of 0.05% polysorbate 80 in phosphate-buffered saline was carcinogenic, producing tumors of the mammary gland in females, osteosarcomas in males, and sarcomas and other related tumors of the peritoneum in both males and females.

TABLE OF CONTENTS

Page

I. Introduction.. 1

II. Materials and Methods.................................... 3

A. Chemical.. 3
B. Dosage Preparation...................................... 3
C. Animals... 4
D. Animal Maintenance...................................... 5
E. Subchronic Studies...................................... 9
F. Designs of Chronic Studies.............................. 10
G. Clinical and Pathologic Examinations.................... 17
H. Data Recording and Statistical Analyses................. 18

III. Results - Rats.. 25

A. Body Weights and Clinical Signs (Rats).................. 25
B. Survival (Rats)... 25
C. Pathology (Rats).. 31
D. Statistical Analyses of Results (Rats).................. 35

IV. Results - Mice... 41

A. Body Weights and Clinical Signs (Mice).................. 41
B. Survival (Mice)... 41
C. Pathology (Mice).. 47
D. Statistical Analyses of Results (Mice).................. 49

V. Discussion.. 51

VI. Bibliography... 57

APPENDIXES

Appendix A Summary of the Incidence of Neoplasms in Rats Given Intraperitoneal Injections of Acronycine.................................... 59

Table A1 Summary of the Incidence of Neoplasms in Male Rats Given Intraperitoneal Injections of Acronycine (Control Groups)........................ 61

Page

Table A2 Summary of the Incidence of Neoplasms in Male Rats Given Intraperitoneal Injections of Acronycine (Treated Groups).................... 64

Table A3 Summary of the Incidence of Neoplasms in Female Rats Given Intraperitoneal Injections of Acronycine (Control Groups).................... 68

Table A4 Summary of the Incidence of Neoplasms in Female Rats Given Intraperitoneal Injections of Acronycine (Treated Groups).................... 71

Appendix B Summary of the Incidence of Neoplasms in Mice Given Intraperitoneal Injections of Acronycine.. 75

Table B1 Summary of the Incidence of Neoplasms in Male Mice Given Intraperitoneal Injections of Acronycine (Control Groups).................... 77

Table B2 Summary of the Incidence of Neoplasms in Male Mice Given Intraperitoneal Injections of Acronycine (Control and Treated Groups)......... 80

Table B3 Summary of the Incidence of Neoplasms in Female Mice Given Intraperitoneal Injections of Acronycine (Control Groups).................... 83

Table B4 Summary of the Incidence of Neoplasms in Female Mice Given Intraperitoneal Injections of Acronycine (Control and Treated Groups)......... 86

Appendix C Summary of the Incidence of Nonneoplastic Lesions in Rats Given Intraperitoneal Injections of Acronycine.......................... 89

Table C1 Summary of the Incidence of Nonneoplastic Lesions in Male Rats Given Intraperitoneal Injections of Acronycine (Control Groups).......... 91

Table C2 Summary of the Incidence of Nonneoplastic Lesions in Male Rats Given Intraperitoneal Injections of Acronycine (Treated Groups).......... 94

Page

Table C3 Summary of the Incidence of Nonneoplastic Lesions in Female Rats Given Intraperitoneal Injections of Acronycine (Control Groups).......... 98

Table C4 Summary of the Incidence of Nonneoplastic Lesions in Female Rats Given Intraperitoneal Injections of Acronycine (Treated Groups).......... 101

Appendix D Summary of the Incidence of Nonneoplastic Lesions in Mice Given Intraperitoneal Injections of Acronycine........................... 105

Table D1 Summary of the Incidence of Nonneoplastic Lesions in Male Mice Given Intraperitoneal Injections of Acronycine (Control Groups).......... 107

Table D2 Summary of the Incidence of Nonneoplastic Lesions in Male Mice Given Intraperitoneal Injections of Acronycine (Control and Treated Groups).................................... 110

Table D3 Summary of the Incidence of Nonneoplastic Lesions in Female Mice Given Intraperitoneal Injections of Acronycine (Control Groups).......... 115

Table D4 Summary of the Incidence of Nonneoplastic Lesions in Female Mice Given Intraperitoneal Injections of Acronycine (Control and Treated Groups).................................... 118

Appendix E Analyses of the Incidence of Primary Tumors in Rats Given Intraperitoneal Injections of Acronycine...................................... 123

Table E1 Time-adjusted Analyses of the Incidence of Primary Tumors in Male Rats Given Intraperitoneal Injections of Acronycine, Using Vehicle Controls... 125

Table E2 Time-adjusted Analyses of the Incidence of Primary Tumors in Female Rats Given Intraperitoneal Injections of Acronycine, Using Vehicle Controls... 133

Appendix F Analyses of the Incidence of Primary Tumors in Mice Given Intraperitoneal Injections of Acronycine.. 145

Page

Table F1 Time-adjusted Analyses of the Incidence of Primary Tumors in Male Mice Given Intraperitoneal Injections of Acronycine.............................. 147

Table F2 Time-adjusted Analyses of the Incidence of Primary Tumors in Female Mice Given Intraperitoneal Injections of Acronycine............. 149

TABLES

Table 1 Design of Chronic Studies of Acronycine in Rats.. 11

Table 2 Design of Chronic Studies of Acronycine in Male Mice....................................... 13

Table 3 Design of Chronic Studies of Acronycine in Female Mice..................................... 15

FIGURES

Figure 1 Growth Curves for Male Rats Treated with Acronycine... 26

Figure 2 Growth Curves for Female Rats Treated with Acronycine... 27

Figure 3 Survival Curves for Male Rats Treated with Acronycine... 28

Figure 4 Survival Curves for Female Rats Treated with Acronycine... 29

Figure 5 Life Table for Male Rats Treated with Acronycine: Osteosarcoma of the Musculoskeletal System................................ 37

Figure 6 Growth Curves for Male Mice Treated with Acronycine... 42

Figure 7 Growth Curves for Female Mice Treated with Acronycine... 43

Page

Figure 8 Survival Curves for Male Mice Treated with Acronycine.. 44

Figure 9 Survival Curves for Female Mice Treated with Acronycine.. 45

I. INTRODUCTION

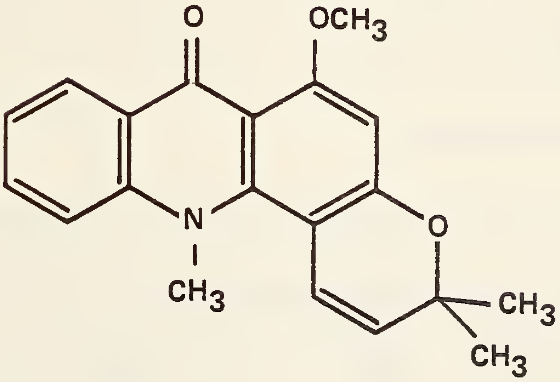
Acronycine (CAS 7008-42-6, NSC 403169, NCI C01536), an alkaloid derived from the bark of the Australian scrub ash (Lahey and Thomas, 1949), has been investigated as an experimental anti-cancer drug. In preclinical screening tests in mice, broad-spectrum antitumor activity of acronycine was demonstrated (Svoboda et al., 1966). Phase I clinical trials were conducted but have not been reported (Carter, 1971). Acronycine inhibits cellular uptake of two extracellular nucleosides (uridine and thymidine) necessary for DNA and RNA synthesis, apparently by interfering with their transport across cell membranes (Dunn et al., 1973).

Acronycine was selected for screening in the carcinogenesis program in an attempt to evaluate the carcinogenicity of certain drugs that may be used for prolonged periods in humans.

II. MATERIALS AND METHODS

A. Chemical

ACRONYCINE



Acronycine, which is the name used most commonly for 3,12-dihydro-6-methoxy-3,3,12-trimethyl-7H-pyrano(2,3-c)acridin-7-one, was obtained through the Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, in two batches that were manufactured by the Commonwealth Scientific and Industrial Research Organization, East Melbourne, Australia. The identity of the chemical was confirmed in analyses performed by Stanford Research Institute for the Developmental Therapeutics Program. These analyses included melting point of the chemical and its picrate salt; elemental analyses (C, H, N) for $C_{20}H_{19}NO_3$; and infrared, ultraviolet, and nuclear magnetic resonance spectra. Thin-layer chromatography showed only trace impurities.

No attempt was made to identify or quantitate these impurities. All data indicate that these batches of acronycine were nearly 100% pure.

The bulk chemical was stored in a brown glass bottle. This bottle was enclosed in a plastic bag containing Drierite® and was refrigerated at 5°C.

B. Dosage Preparation

Test solutions were prepared daily by adding a specified amount of the drug to a vehicle composed of 0.05% polysorbate 80 in phosphate-buffered saline. This mixture was emulsified in a 10-ml Potter-Elvehjem tissue grinder with a Teflon pestle for 20 seconds. Each concentration (0.02, 0.06, 0.125, 0.15, 0.25, 0.3, or 0.6%) was administered on the day of preparation.

C. Animals

Sprague-Dawley rats obtained from Charles River Breeding Laboratories, Inc., Wilmington, Massachusetts, were used for all groups of this species. B6C3F1 mice obtained from A. R. Schmidt, Madison, Wisconsin, were used for the upper mid- and high-dose groups and respective controls; B6C3F1 mice from Charles River Laboratories, for the lower mid-dose groups and controls; and B6C3F1 mice from Litton Bionetics, Frederick, Maryland, for the low-dose groups and controls. The rats used in the chronic studies were 30-42 days old on arrival at the laboratory; the mice were 30-32 days old. All animals were quarantined (rats: 5 days in the original study, 12 days in the rerun; mice: 5 days in the original study, 10 days in the first rerun, 13 days in the second rerun). Animals having no visible signs of disease were assigned to control and treated groups and earmarked for individual identification.

D. Animal Maintenance

Animals were placed on study at different intervals during a 4-year period. Some techniques for animal care were improved during this time, and as a result, the animal groups placed on study at the beginning of the bioassay (high- and mid-dose rats, high- and upper mid-dose mice) were exposed to somewhat different environmental conditions than the groups started later (low-dose rats; lower mid- and low-dose mice).

During all of the studies, animals were housed in temperature- and humidity-controlled rooms. The temperature range was 20-24°C, and the relative humidity was maintained at 40-60%. The room air was changed 15 times per hour and passed through both intake and exhaust fiberglass roughing filters. In addition to natural light, illumination was provided by fluorescent light for

9 hours per day. Wayne® Lab Blox animal meal (Allied Mills, Inc., Chicago, Ill.) and water were supplied daily and were available ad libitum.

All animals were housed in solid-bottom stainless steel cages (Hahn Roofing and Sheet Metal Co., Birmingham, Ala.). Rats were housed five per cage, and mice in the original groups (high- and upper mid-dose) were housed seven per cage; mice in later groups (lower mid- and low-dose) were housed five per cage, due to a reduction in cage size. The bottoms of the rat cages were lined with Iso-Dri® hardwood chips (Carworth, Edison, N. J.), and cage tops were covered with disposable filter bonnets beginning at week 34 for the high- and mid-dose groups of rats and at week 1 for the low-dose group of rats.

Mouse cages were provided with Sterolit® clay bedding (Englehard Mineral and Chemical Co., New York, N. Y.), except for the cages of the low-dose mice, which were provided with Betta-Chip® hardwood bedding (Northeastern Products Corp., Warrensburg, N.Y.) from week 84 until termination of the study. Filter bonnets were installed on cages of the low-dose mice in week 32.

Bedding was replaced once per week; cages, water bottles, and feeders were sanitized at 82°C once per week; and racks were cleaned once per week, except during the later studies with low-dose rats and low-dose mice, when clean cages and fresh bedding were provided twice per week.

Rats and mice were housed in separate rooms. Control animals were housed with respective treated animals. Animals treated with acronycine were maintained in the same rooms as animals of the same species being treated with the following chemicals:

RATS

Gavage Studies

cholesterol (p-(bis(2-chloroethyl)amino)phenyl)acetate (phenesterin) (CAS 3546-10-9)
estradiol bis((p-(bis(2-chloroethyl)amino)phenyl)acetate) (estradiol mustard) (CAS 22966-79-6)

Intraperitoneal Injection Studies

4′-(9-acridinylamino)methansulfon-m-aniside monohydrochloride (MAAM) (NSC 141549)
5-azacytidine (CAS 320-67-2)
beta-2′-deoxy-6-thioguanosine monohydrate (beta-TGdR) (CAS 789-61-7)
1,4-butanediol dimethanesulfonate (busulfan) (CAS 55-98-1)
emetine dihydrochloride tetrahydrate (CAS 316-42-7)
3,3′-iminobis-1-propanol dimethanesulfonate (ester) hydrochloride [IPD] (CAS 3458-22-8)
(±)-4,4′-(1-methyl-1,2-ethanediyl)bis-2,6-piperazinedione (ICRF-159) (CAS 21416-87-5)
N,3-bis(2-chloroethyl)tetrahydro-2H-1,3,2-oxazaphosphorin-2-amine-2-oxide (isophosphamide) (CAS 3778-73-2)
N-(2-chloroethyl)-N-(1-methyl-2-phenoxyethyl)benzylamine hydrochloride (phenoxybenzamine hydrochloride) (CAS 63-92-3)
N-(1-methylethyl)-4-((2-methylhydrazino)methyl)benzamide monohydrochloride (procarbazine) (CAS 366-70-1)
tris(1-aziridinyl)phosphine sulfide (thio-TEPA) (CAS 52-24-4)
2,4,6-tris(dimethylamino)-s-triazine (CAS 645-05-6)

MICE

Feed Studies

4-acetyl-N-((cyclohexylamino)carbonyl)benzenesulfonamide (acetohexamide) (CAS 968-81-0)
anthranilic acid (CAS 118-92-3)
1-butyl-3-(p-tolylsulfonyl)urea (tolbutamide) (CAS 64-77-7)
4-chloro-N-((propylamino)carbonyl)benzenesulfonamide (chlorpropamide) (CAS 94-20-2)
5-(4-chlorophenyl)-6-ethyl-2,4-pyrimidinediamine (pyrimethamine) (CAS 58-14-0)
2,6-diamino-3-(phenylazo)pyridine hydrochloride (phenazopyridine hydrochloride) (CAS 136-40-3)
L-tryptophan (CAS 73-22-3)
N-9H-fluoren-2-ylacetamide (CAS 53-96-3)
N-(p-toluenesulfonyl)-N′-hexamethyleniminourea (tolazamide) (CAS 1156-19-0)
1-phenethylbiguanide hydrochloride (phenformin) (CAS 114-86-3)
pyrazinecarboxamide (pyrazinamide) (CAS 98-96-4)
4,4′-sulfonyldianiline (dapsone) (CAS 80-08-0)
4,4′-thiodianiline (CAS 139-65-1)
ethionamide (CAS 536-33-4)
reserpine (CAS 50-55-5)

Gavage Studies

cholesterol (p-(bis(2-chloroethyl)amino)phenyl)acetate (phenesterin) (CAS 3546-10-9)
estradiol bis((p-(bis(2-chloroethyl)amino)phenyl)acetate) (estradiol mustard) (CAS 22966-79-6)

Intraperitoneal Injection Studies

4′-(9-acridinylamino)methansulfon-m-aniside monohydrochloride (MAAM) (NSC 141549)
5-azacytidine (CAS 320-67-2)
beta-2′-deoxy-6-thioguanosine monohydrate (beta-TGdR) (CAS 789-61-7)
1,4-butanediol dimethanesulfonate (busulfan) (CAS 55-98-1)
emetine dihydrochloride tetrahydrate (CAS 316-42-7)
3,3′-iminobis-1-propanol dimethanesulfonate (ester) hydrochloride [IPD] (CAS 3458-22-8)
(±)-4,4′-(1-methyl-1,2-ethanediyl)bis-2,6-piperazinedione (ICRF-159) (CAS 21416-87-5)
N,3-bis(2-chloroethyl)tetrahydro-2H-1,3,2-oxazaphosphorin-2-amine-2-oxide (isophosphamide) (CAS 3778-73-2)

N-(2-chloroethyl)-N-(1-methyl-2-phenoxyethyl)benzylamine
  hydrochloride (phenoxybenzamine hydrochloride) (CAS 63-92-3)
N-(1-methylethyl)-4-((2-methylhydrazino)methyl)benzamide
  monohydrochloride (procarbazine) (CAS 366-70-1)
tris(1-aziridinyl)phosphine sulfide (thio-TEPA) (CAS 52-24-4)
2,4,6-tris(dimethylamino)-s-triazine (CAS 645-05-6)
hexamethylmelamine (CAS 148-82-3)

E. Subchronic Studies

Subchronic studies were conducted to estimate the maximum tolerated doses of acronycine, on the basis of which low and high concentrations (hereinafter referred to as "low doses" and "high doses") were determined for administration in the chronic studies using one or both sexes of each species. In subchronic studies of acronycine, male and female Sprague-Dawley rats and male Swiss mice were administered the test chemical by intraperitoneal injection three times per week for 45 days at one of five different doses. Following administration of the chemical, all surviving animals were observed for an additional 45 days. Treated groups each consisted of five animals, untreated-control groups consisted of 10 animals, and vehicle control (0.05% polysorbate in buffered saline) groups consisted of 10 animals. All animals were observed daily and weighed once per week.

The first subchronic study was on female rats using 60, 150, 300, 600, or 1,200 mg/kg body weight for each injection. Weight depression and deaths occurred at all doses. Thus, a second study was performed, using 1.5, 3.75, 7.5, 15, or 30 mg/kg. Male Sprague-Dawley rats which were available at the time were used. Four animals receiving 30 mg/kg died, but no deaths occurred in the remaining four groups. Mean body weights were depressed during the period of chemical administration, but the animals recovered and no weight depression greater than the 15% limit was present at day 90. No gross abnormalities were observed. Low and high doses for chronic studies using rats were set at 7.5 and 15 mg/kg.

All mice treated at the doses originally selected (100, 250, 500, 1,000, or 2,000 mg/kg) died by week 6. A second study was performed using doses of 2.5, 6.25, 12.5, 25, or 50 mg/kg. By day 90, only one animal treated at a dose of 50 mg/kg had survived. No deaths occurred in the groups receiving 2.5, 6.25, or 25 mg/kg, although one animal treated at 12.5 mg/kg died during week 8. Weight gains were not affected in these latter four groups, and no gross abnormalities were observed at necropsy. Low and high doses for chronic studies using mice were set at 12.5 and 25 mg/kg.

F. Designs of Chronic Studies

The designs of the chronic studies are shown in tables 1, 2, and 3.

Table 1. Design of Chronic Studies of Acronycine in Rats

Sex and Test Group	Initial No. of Animals[a]	Acronycine Dose[b] (mg/kg)	Time on Study	
			Treated (weeks)	Untreated (weeks)
Male				
Low-Dose Untreated-Control[c]	10	0		81
Low-Dose Vehicle-Control[c]	10	0[d]	52	28
Low-Dose[c]	35	3.75	52	28
Mid- and High-Dose Untreated-Control	10	0		82
Mid- and High-Dose Vehicle-Control	10	0[d]	52	30
Mid-Dose	35	7.5	52	29
High-Dose	35	15	51[e]	
Female				
Low-Dose Untreated-Control[c]	10	0		80
Low-Dose Vehicle-Control[c]	10	0[d]	52	28
Low-Dose[c]	35	3.75	52	28
Mid- and High-Dose Untreated Control	10	0		82
Mid- and High-Dose Vehicle Control	10	0[d]	52	30
Mid-Dose	35	7.5	52	29-30
High-Dose	35	15	52	28-29

[a] Ages of rats when placed on study: mid- and high-dose males, 40 days; mid- and high-dose females, 47 days; low-dose males and females, 42 days.

[b] Acronycine was administered intraperitoneally in a vehicle consisting of polysorbate 80 in phosphate-buffered saline at a volume of 0.25 ml/100 g body weight three times per week; doses were based on individual weights. The same needle for injection was used for each group of five animals within a cage.

Table 1. Design of Chronic Studies of Acronycine in Rats

(continued)

[c] Because of high mortality in treated groups, new treated and control groups were started 77 weeks after the original start of the study.

[d] Vehicle controls received only polysorbate 80 in phosphate-buffered saline at the same volume as the treated animals. The same bottle of vehicle solution was used for all vehicle-control animals on study at any given time.

[e] All high-dose males died or were killed by week 51.

Table 2. Design of Chronic Studies of Acronycine in Male Mice

Sex and Test Group	Initial No. of Animals[a]	Acronycine Dose[b] (mg/kg)	Time on Study	
			Treated (weeks)	Untreated (weeks)
Low-Dose Untreated-Control[c]	20	0		105
Low-Dose Vehicle-Control[c]	20	0[d]	71[e]	
Low-Dose[c]	40	2	92[e]	
Lower Mid-Dose Untreated-Control[f]	10	0		79
Lower Mid-Dose Vehicle-Control[f]	10	0[d]	52	26
Lower Mid-Dose[f]	35	6	49[e]	
Upper Mid-Dose and High-Dose Untreated Control	10	0		78
Upper Mid-Dose and High-Dose Vehicle Control	10	0[d]	31	47
Upper Mid-Dose	35	12.5	31	14
High-Dose	35	25	25[e]	

[a] Ages of mice when placed on study: upper mid-dose and high-dose 35 days; lower mid-dose, 42 days; low-dose, 43 days.

[b] Acronycine was administered intraperitoneally in a vehicle consisting of polysorbate 80 in phosphate-buffered saline at a volume of 1 ml/100 g body weight three times per week; doses were based on the mean weight of the animals in each cage. The same needle for injection was used for each group of five animals (restarted groups) or seven animals (original groups) within a cage.

[c] Because of high mortality in the treated animals, treated and control groups, designated "low-dose," were started 97 weeks after the original start of the study.

[d] Vehicle controls received only polysorbate 80 in phosphate-buffered saline at the same volume as the treated animals. The same bottle of vehicle solution was used for all vehicle-control animals on study at any given time.

Table 2 Design of Chronic Studies of Acronycine in Male Mice

(continued)

---

[e] All animals died or were killed by the times indicated.

[f] Because of high mortality in the treated animals, treated and control groups, designated "lower mid-dose," were started 46 weeks after the original start of the study.

Table 3. Design of Chronic Studies of Acronycine in Female Mice

Sex and Test Group	Initial No. of Animals[a]	Acronycine Dose[b] (mg/kg)	Time on Study	
			Treated (weeks)	Untreated (weeks)
Low-Dose Untreated-Control[c]	20	0		105
Low-Dose Vehicle-Control[c]	20	0[d]	56[e]	
Low-Dose[c]	40	2	87[e]	
Lower Mid-Dose Untreated-Control[f]	10	0		79
Lower Mid-Dose Vehicle-Control[f]	10	0[d]	52	26
Lower Mid-Dose[f]	35	6	49[e]	
Upper Mid-Dose and High-Dose Untreated Control	10	0		79
Upper Mid-Dose and High-Dose Vehicle Control	10	0[d]	31	47
Upper Mid-Dose	35	12.5	31	10[e]
High-Dose	35	25	25[e]	

[a] Ages of mice when placed on study: upper mid-dose and high-dose, 35 days; lower mid-dose, 42 days; low-dose, 43 days.

[b] Acronycine was administered intraperitoneally in a vehicle consisting of polysorbate 80 in phosphate-buffered saline at a volume of 1 ml/100 g body weight three times per week; doses were based on mean weight of the animals in each cage. The same needle for injection was used for each group of five animals (restarted groups) or seven animals (original groups) within a cage.

[c] Because of high mortality in the treated animals, treated and control groups, designated "low-dose," were started 97 weeks after the original start of the study.

[d] Vehicle controls received only polysorbate 80 in phosphate-buffered saline at the same volume as the treated animals. The same bottle of vehicle solution was used for all vehicle-control animals on study at any given time.

Table 3. Design of Chronic Studies of Acronycine in Female Mice

(continued)

---

[e] All animals died or were killed by the times indicated.

[f] Because of high mortality in the treated animals, treated and control groups, designated "lower mid-dose," were started 46 weeks after the original start of the study.

G. Clinical and Pathologic Examinations

All animals were observed twice daily for signs of toxicity, and animals that were moribund were killed and necropsied. The animals were weighed individually each week, every 2 weeks, or once per month, depending on the schedule in use at the time the animals were weighed. Palpation for masses was carried out at each weighing.

The pathologic evaluation consisted of gross and microscopic examination of major tissues, major organs, and all gross lesions from killed animals and from animals found dead. The following tissues were examined microscopically: skin, muscle, lungs and bronchi, trachea, bone and bone marrow, spleen, lymph nodes, thymus, heart, salivary gland, liver, gallbladder and bile duct (mice), pancreas, esophagus, stomach, small intestine, large intestine, kidney, urinary bladder, pituitary, adrenal, thyroid, parathyroid, mammary gland, prostate or uterus, testis or ovary, brain, and sensory organs. Peripheral blood smears were prepared from each animal killed. Occasionally, additional tissues were also examined microscopically. The different tissues were preserved in 10% buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Special staining techniques were utilized when indicated for more definitive diagnosis.

A few tissues from some animals were not examined, particularly from those animals that died early. Also, some animals were missing, cannibalized, or judged to be in such an advanced state of autolysis as to preclude histopathologic evaluation. Thus, the number of animals from which particular organs or tissues were examined microscopically varies, and does not necessarily represent the number of animals that were placed on study in each group.

H. Data Recording and Statistical Analyses

Pertinent data on this experiment have been recorded in an automatic data processing system, the Carcinogenesis Bioassay Data System (Linhart et al., 1974). The data elements include descriptive information on the chemicals, animals, experimental design, clinical observations, survival, body weight, and individual pathologic results, as recommended by the International Union Against Cancer (Berenblum, 1969). Data tables were generated for verification of data transcription and for statistical review.

These data were analyzed using the statistical techniques described in this section. Those analyses of the experimental results that bear on the possibility of carcinogenicity are discussed in the statistical narrative sections.

Probabilities of survival were estimated by the product-limit procedure of Kaplan and Meier (1958) and are presented in this report in the form of graphs. Animals were statistically censored as of the time that they died of other than natural causes or were found to be missing; animals dying from natural causes were not statistically censored. Statistical analyses for a possible dose-related effect on survival used the method of Cox (1972) for testing two groups for equality and Tarone′s (1975) extensions of Cox′s methods for testing for a dose-related trend. One-tailed P values have been reported for all tests except the departure from linearity test, which is only reported when its two-tailed P value is less than 0.05.

The incidence of neoplastic or nonneoplastic lesions has been given as the ratio of the number of animals bearing such lesions at a specific anatomic site (numerator) to the number of animals in which that site is examined (denominator). In most instances, the denominators included only those animals for which that site was examined histologically. However, when macroscopic examination was required to detect lesions prior to histologic sampling (e.g., skin or mammary tumors), or when lesions could have appeared at multiple sites (e.g., lymphomas), the denominators consist of the numbers of animals necropsied.

The purpose of the statistical analyses of tumor incidence is to determine whether animals receiving the test chemical developed a significantly higher proportion of tumors than did the control animals. As a part of these analyses, the one-tailed Fisher exact test (Cox, 1970) was used to compare the tumor incidence of a control group with that of a group of treated animals at each dose level. When results for a number of treated groups (k) are compared simultaneously with those for a control group, a correction to ensure an overall significance level of 0.05 may be made. The Bonferroni inequality (Miller, 1966) requires that the P value for any comparison be less than or equal to 0.05/k. In cases where this correction was used, it is discussed in the narrative section. It is not, however, presented in the tables, where the Fisher exact P values are shown.

The Cochran-Armitage test for linear trend in proportions, with continuity correction (Armitage, 1971), was also used. Under the assumption of a linear trend, this test determines if the slope of the dose-response curve is different from zero at the one-tailed 0.05 level of significance. Unless otherwise noted, the direction of the significant trend is a positive dose relationship. This method also provides a two-tailed test of departure from linear trend.

A time-adjusted analysis was applied when numerous early deaths resulted from causes that were not associated with the formation of tumors. In this analysis, deaths that occurred before the first tumor was observed were excluded by basing the statistical tests on animals that survived at least 52 weeks, unless a tumor was found at the anatomic site of interest before week 52. When such an early tumor was found, comparisons were based exclusively on animals that survived at least as long as the animal in which the first tumor was found. Once this reduced set of data was obtained, the standard procedures for analyses of the incidence of tumors (Fisher exact tests, Cochran-Armitage tests, etc.) were followed.

When appropriate, life-table methods were used to analyze the incidence of tumors. Curves of the proportions surviving without an observed tumor were computed as in Saffiotti et al. (1972). The week during which an animal died naturally or was sacrificed was entered as the time point of tumor observation. Cox's methods of comparing these curves were used for two groups; Tarone's extension to testing for linear trend was used for three groups. The statistical tests for the incidence of tumors which used life-table methods were one-tailed and, unless otherwise noted, in the direction of a positive dose relationship. Significant departures from linearity ($P < 0.05$, two-tailed test) were also noted.

The approximate 95 percent confidence interval for the relative risk of each treated group compared to its control was calculated from the exact interval on the odds ratio (Gart, 1971). The relative risk is defined as p_t/p_c where p_t is the true binomial probability of the incidence of a specific type of tumor in a treated group of animals and p_c is the true probability of the spontaneous incidence of the same type of tumor in a control group. The hypothesis of equality between the true proportion of a specific tumor in a treated group and the proportion in a control group corresponds to a relative risk of unity. Values in excess of unity represent the condition of a larger proportion in the treated group than in the control.

The lower and upper limits of the confidence interval of the relative risk have been included in the tables of statistical analyses. The interpretation of the limits is that in approximately 95% of a large number of identical experiments, the true ratio of the risk in a treated group of animals to that in a control group would be within the interval calculated from the experiment. When the lower limit of the confidence interval is greater than one, it can be inferred that a statistically significant result ($P < 0.025$ one-tailed test when the control incidence is not zero, $P < 0.050$ when the control incidence is zero) has occurred. When the lower limit is less than unity, but the upper limit is greater than unity, the lower limit indicates the absence of a significant result while the upper limit indicates that there is a theoretical possibility of the induction of tumors by the test chemical, which could not be detected under the conditions of this test.

III. RESULTS - RATS

A. Body Weights and Clinical Signs (Rats)

Mean body weights of both male and female rats at all doses were lower than those of the vehicle and untreated controls during most of the study (figures 1 and 2). Fluctuations in the growth curve may be due to mortality; as the size of the group diminishes, the mean body weight may be subject to wide variation.

Rales were noted in a few animals of both treated and control groups. No other clinical signs were recorded that could be related to toxicity or early deaths. To control respiratory disease, the mid- and high-dose groups and respective controls received oxytetracycline in the drinking water at 0.6 mg/ml during weeks 34 to 40 and at 0.3 mg/ml during weeks 40 to 44.

B. Survival (Rats)

The Kaplan and Meier curves estimating the probabilities of survival for male and female rats administered acronycine by injection at the doses of this experiment, together with the untreated and vehicle controls, are shown in figures 3 and 4.

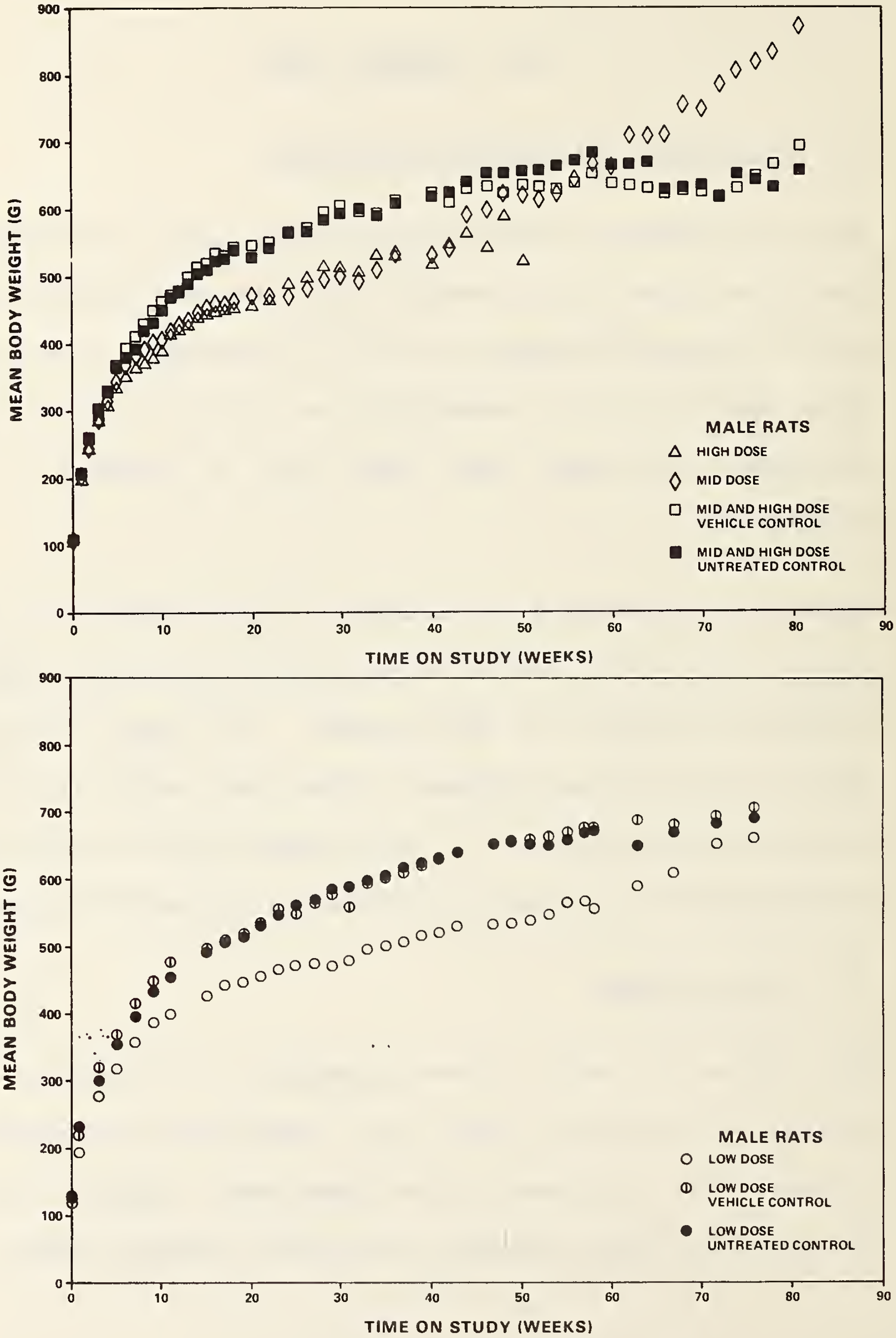


Figure 1. Growth Curves for Male Rats Treated With Acronycine

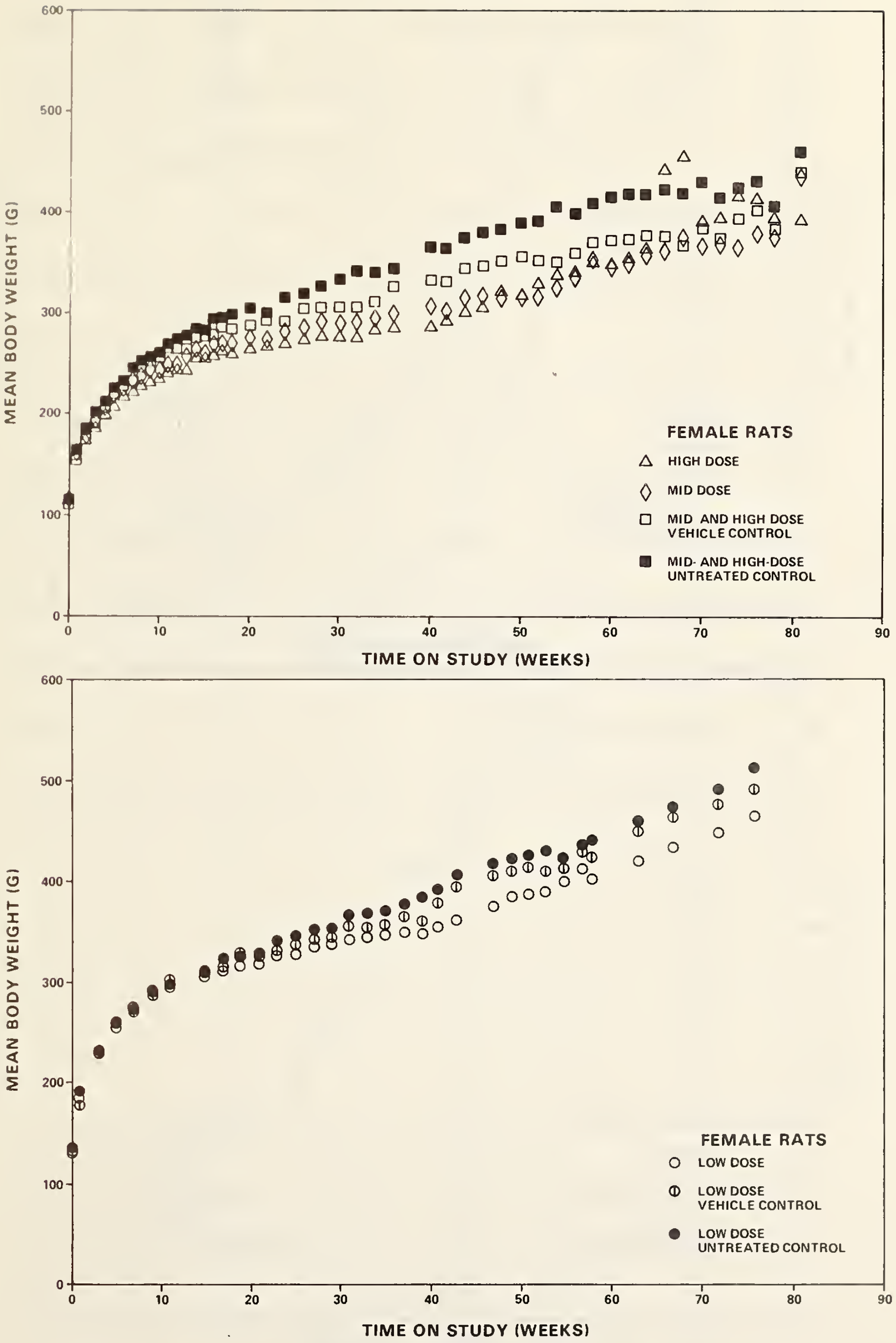


Figure 2. Growth Curves for Female Rats Treated with Acronycine

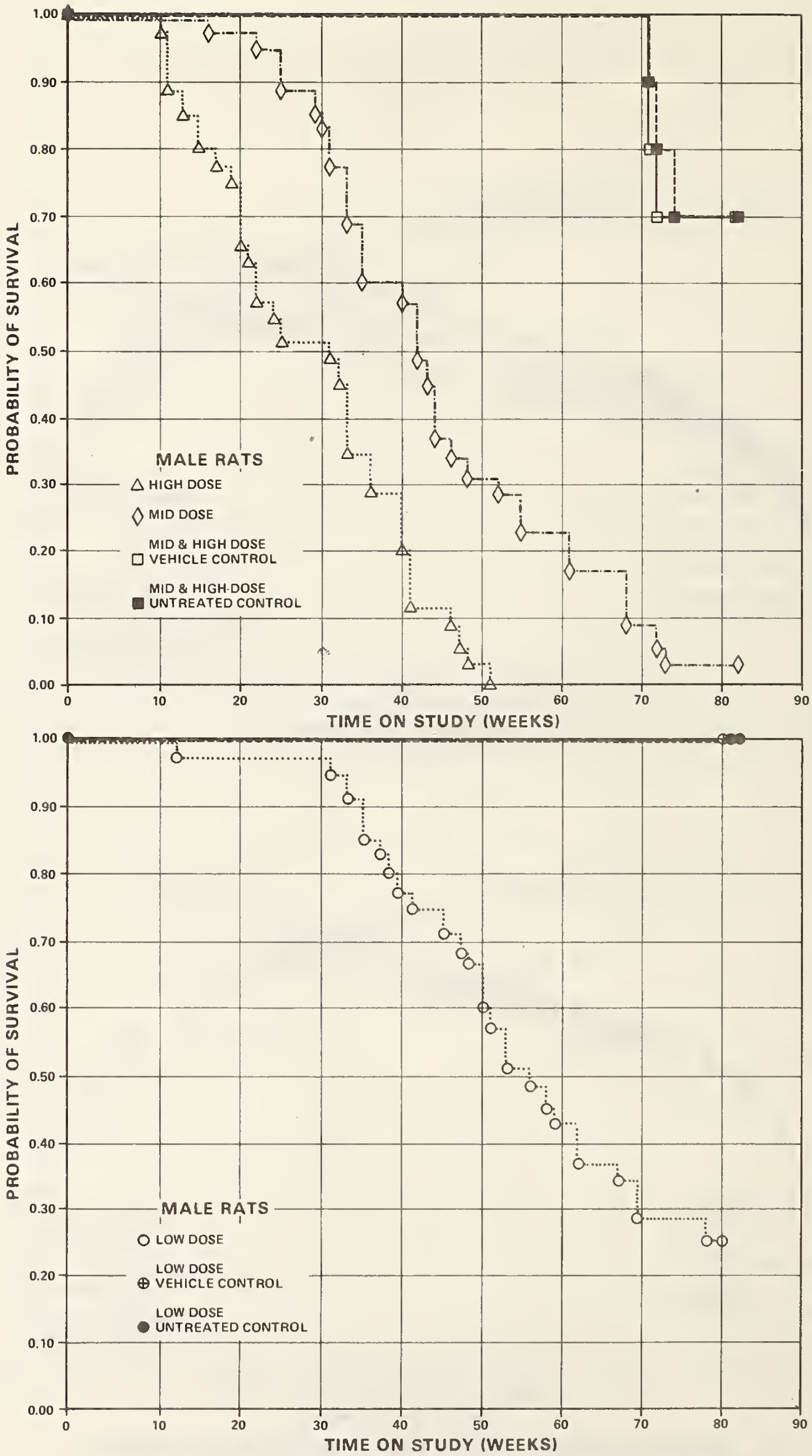
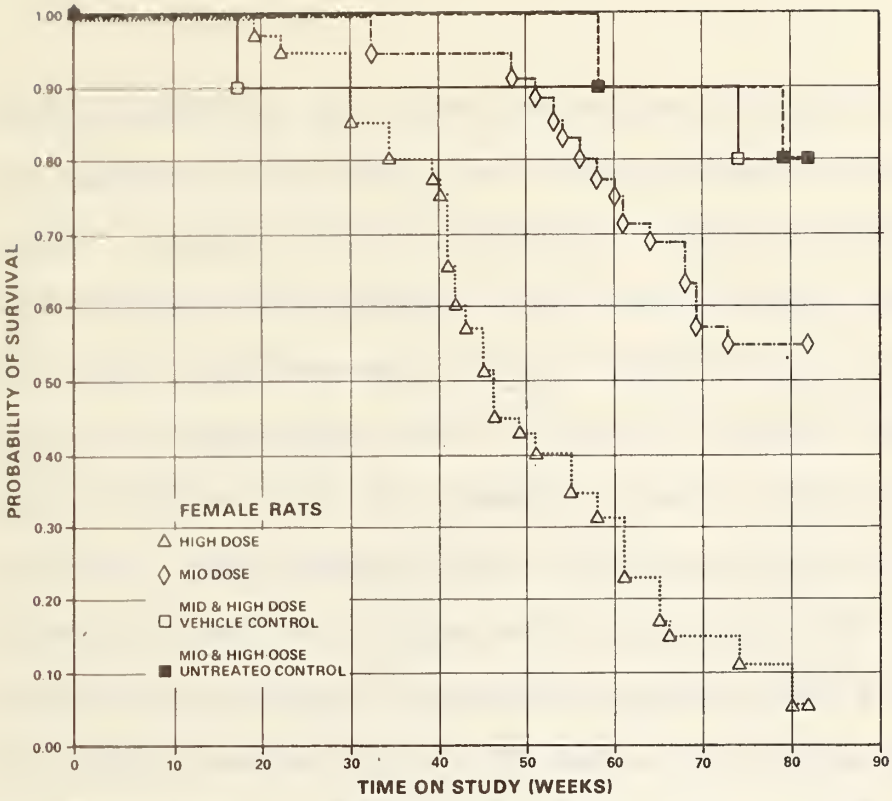


Figure 3. Survival Curves for Male Rats Treated with Acronycine



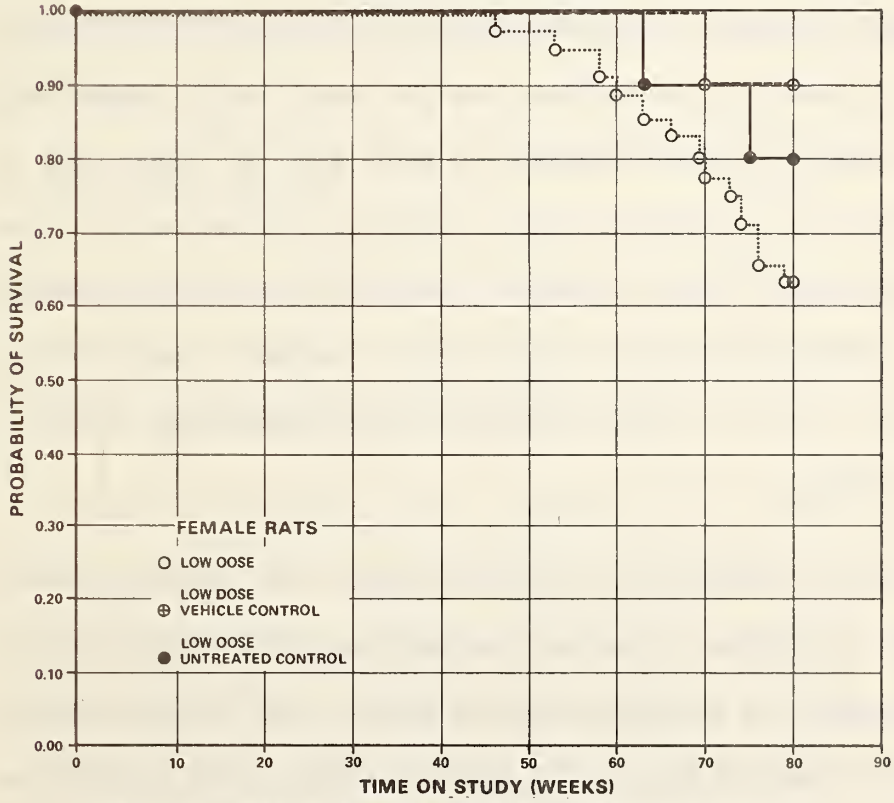


Figure 4. Survival Curves for Female Rats Treated with Acronycine

In male rats, the results of the Tarone test for positive dose-related trend in mortality over the period of the bioassay are significant ($P < 0.001$), using either set of controls. Also, each of the treated groups has a significantly lower survival than either control group. In the high-dose group, only 2/35 (6%) animals survived to week 47 of the study, and the median time on study was 31 weeks; however, the first observed tumors occurred as early as week 32. In the mid-dose group, 1/35 (3%) animals survived to the end of the study, 9/35 (31%) survived to week 52, the median time on study was 42 weeks, and the first observed tumor occurred at week 35. In the low-dose group, 9/35 (26%) animals survived to termination of the study, 20/35 (57%) survived to week 52, the median time on study was 56 weeks, and the first observed tumor occurred at week 48. At least 70% of the animals in the control groups (10/10 in either set of low-dose controls and 7/10 in either set of mid- and high-dose controls) lived to the end of the study. The early deaths of the male treated rats may have suppressed the incidences of late-appearing tumors.

In female rats, the results of the Tarone test are significant ($P < 0.001$), using the high-dose, the mid-dose, and either set of control groups, and an indicated departure from linear trend is observed ($P = 0.008$), due to the steep increase in deaths in the female high-dose rats. The survival of the low-dose group did not differ significantly from that of either of its control groups. In the high-dose group, only 4/35 (11%) animals survived to week 80, 14/35 (40%) survived to week 52, the median time on study was 46 weeks, and the first observed tumor occurred at week 39. In the mid-dose group 19/35 (54%), in the low-dose group 23/35 (66%), and in the controls at least 80% of the animals (9/10 of the low-dose vehicle controls, 8/10 of the mid- and high-dose vehicle controls, 8/10 of the low-dose untreated controls, and 8/10 of the mid- and high-dose untreated controls) survived to termination of the study. The early deaths of the high-dose female rats may have suppressed the incidences of late-appearing tumors.

C. Pathology (Rats)

Histopathologic findings on neoplasms in rats are summarized in Appendix A, tables A1-A4; findings on nonneoplastic lesions are summarized in Appendix C, tables C1-C4.

A variety of neoplasms were observed in the control and treated rats. There was a high incidence of neoplasms observed in the treated rats when compared with the untreated- or vehicle-control rats. The treated female rats had a higher incidence of adenocarcinoma of the mammary gland than the control female rats.

In the treated male and female rats, there were malignant neoplasms of mesenchymal tissue, especially of the peritoneal cavity; these included poorly differentiated sarcomas, fibrosarcomas, hemangiosarcomas, malignant mesotheliomas, and osteosarcomas. Similar neoplasms were not observed in any of the control rats.

Malignant neoplasms of the mammary gland were observed in seven low-dose, five mid-dose, and two high-dose female rats. No malignant mammary neoplasms were observed in the control females. The reverse dose relationship of these neoplasms was likely due to the higher number of early deaths and killed moribund animals which occurred in the mid- and high-dose groups. Although the malignant mammary neoplasms varied in histologic appearance, they were classified as adenocarcinomas. These neoplasms were highly cellular and were characterized as focal proliferations of hyperchromatic glandular epithelium. The proliferating epithelium formed small nests and acini which were supported by a fibrous stroma. Papillary proliferation of the mammary epithelium was observed in one of the adenocarcinomas, and large cystic areas were present in a second adenocarcinoma.

A high incidence of osteosarcoma (low-dose 3/31, mid-dose 13/32, high-dose 12/34) was observed in the treated male rats. Most of these neoplasms were observed grossly as enlargements involving the long bones of the limbs. Two osteosarcomas involved vertebrae. Occasionally, the neoplasm appeared to involve only soft tissues, and primary bone involvement was not observed. The osteosarcomas were characterized as anaplastic spindle-cell neoplasms which were forming varying amounts of osteoid. Several of the osteosarcomas had metastasized to other organs, most frequently, to the lung and the liver.

Other types of malignant mesenchymal neoplasms, especially of tissues of the peritoneal cavity, were observed frequently in treated male and female rats. Although all of these neoplasms were poorly differentiated spindle-cell tumors, they were variable in histologic appearance.

Some of the neoplasms were undifferentiated and composed of very pleomorphic spindle cells. These neoplasms were highly cellular and contained undifferentiated mesenchymal cells, poorly differentiated spindle cells, and multinucleated giant cells. The neoplasms were rapidly proliferating, and contained numerous mitotic figures. These undifferentiated sarcomas were classified as sarcomas, NOS (not otherwise specified).

Other poorly differentiated neoplasms appeared to be composed of malignant fibroblasts which were producing varying amounts of collagen. These neoplasms were classified as fibrosarcomas.

A third group of malignant mesenchymal neoplasms found in the treated rats were forming clefts and blood-filled spaces lined by pleomorphic, hyperchromatic endothelial cells. These neoplasms were classified as hemangiosarcomas.

A fourth type of neoplasm observed in the treated rats was classified as mesothelial sarcoma (malignant mesothelioma). These neoplasms were nodular growths arising from the serous membranes lining the peritoneal cavity. They were characterized as papillary projections consisting of a fibrous core covered by large mesothelial cells.

The mesenchymal neoplasms described above appeared to be highly malignant, as evidenced by a high incidence of invasion into adjacent organs and soft tissues and/or metastasis to other sites. Many of these neoplasms were generalized and involved the serosal surfaces of the abdominal viscera.

A variety of nonneoplastic lesions were present in both treated and control animals. The only lesion which appeared to be related to the injection was chronic inflammation in the peritoneal cavity, involving the serosal surfaces of the mesentery and visceral organs. There were also focal areas of coagulative necrosis observed in the liver. These lesions occurred in one vehicle-control rat and several treated rats.

In the judgment of the pathologists, acronycine, at the doses used in this bioassay, induced malignant neoplasms in both male and female rats. Adenocarcinoma of the mammary gland in female rats and malignant neoplasms of mesenchymal tissues in both male and female rats were observed only in the treated groups.

D. Statistical Analyses of Results (Rats)

Tables E1 and E2 in Appendix E contain the time-adjusted statistical analyses of the incidences of those primary tumors that were observed in at least two animals in one group and with an incidence of at least 5% in one or more than one group. Time-adjusted analyses eliminate animals that died before week 52 on study unless a tumor was found at the specific site before this time; in the latter instance, the analysis is based on animals that survived at least as long as the animal in which the first tumor was found. The untreated controls are not included in the tables and the analyses, since the test conditions of the vehicle controls more closely resembled those of the treated animals.

In male rats, the result of the Cochran-Armitage test for positive dose-related trend in the incidence of osteosarcoma of the musculoskeletal system is significant ($P = 0.019$), using the mid- and high-dose vehicle-control group, the mid-dose group, and the high-dose group, and the results of the Fisher exact test show that the incidences in the mid- and high-dose groups are significantly higher than that in the vehicle-control group ($P = 0.027$ and $P = 0.013$, respectively); however, the probability level in the mid-dose group is above the 0.025 level for significance required by the multiple comparison criterion. The life table of the incidence of this tumor in the male rats is shown in figure 5. The result of the Tarone test is significant ($P < 0.001$) when the mid- and high-dose groups are used with their designated control group; however, the result of the Cox test comparing the low-dose group and its vehicle-control group is not significant. The statistical conclusion is that the incidence of osteosarcoma of the musculoskeletal system in male rats is associated with the administration of acronycine. No such tumor was observed in female rats.

Two osteosarcomas of the liver were found in the high-dose male rats. The result of the Cochran-Armitage test on the incidence of this tumor is significant ($P = 0.048$), using the mid- and high-dose vehicle-control group, the mid-dose group, and the high-dose group; however, the results of the Fisher exact test are not significant. Results of statistical tests on the incidences of this tumor in female rats are not significant.

When osteosarcomas of all sites are considered together, the

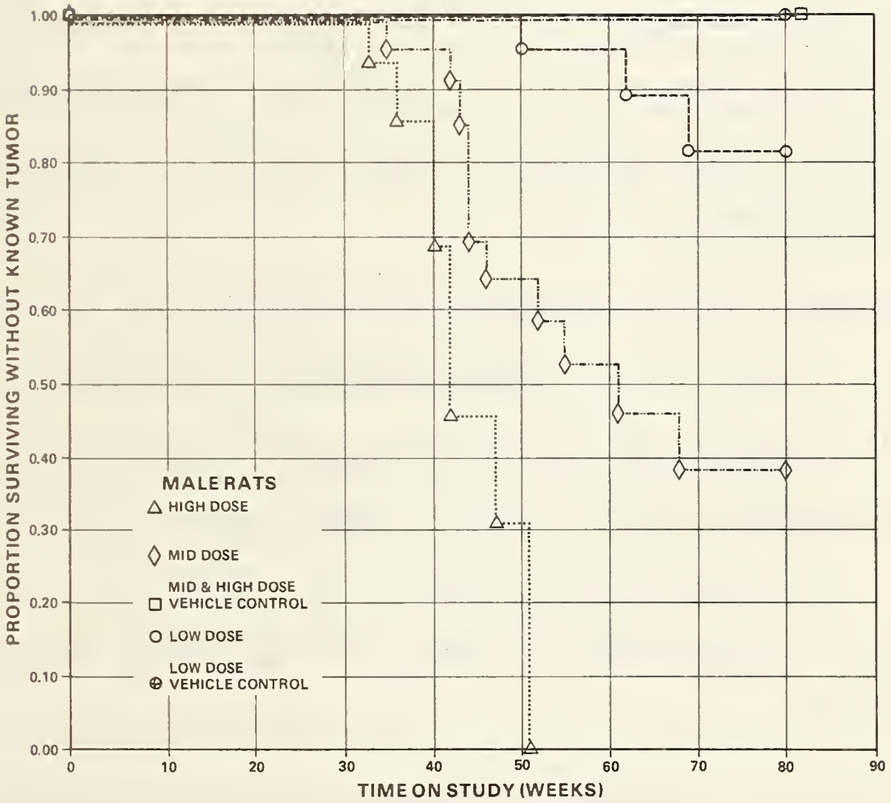


Figure 5. Life Table for Male Rats Treated with Acronycine: Osteosarcoma of the Musculoskeletal System

result of the Cochran-Armitage test is significant (P = 0.002) in the male rats, using the mid- and high-dose vehicle-control group, the mid-dose group, and the high-dose group. The results of the Fisher exact test indicate that the incidences in both the mid- and high-dose groups are significantly higher than that in the control group (P = 0.022 and P = 0.002, respectively). The statistical conclusion is that the incidence of osteosarcomas at all sites in male rats is dose associated. Results of statistical tests on the incidences of these tumors in female rats are not significant.

The result of the Cochran-Armitage test on the incidence of cortical adenoma of the adrenal in male rats is significant (P = 0.045), using the mid- and high-dose vehicle-control group, the mid-dose group, and the high-dose group, but the results of the Fisher exact test are not significant. Results of statistical tests on the incidences of this tumor in female rats are not significant.

The results of the Fisher exact test show that the incidence of fibroadenoma of the mammary gland in low-dose female rats is significantly higher (P = 0.007) than that in the low-dose vehicle controls; however, the incidences of the tumor in the mid- and high-dose groups are not significant. When all tumors of the mammary gland, except fibroma, are combined for analysis, the results of the Fisher exact test show that the incidence in the low-dose group is significantly higher (P = 0.004) than that in the low-dose vehicle controls; however, the result of the Cochran-Armitage test using the mid- and high-dose groups and the appropriate control indicates a significant trend (P = 0.034) in the negative direction. This significant negative trend is due, principally, to the lower incidence observed in the high-dose group. The life-table analysis made using the times of observations of this tumor also yielded a significant negative trend (P = 0.017). As shown in the section concerning survival of the female rats, the high-dose group evidences a steep decrease in survival compared with the other groups.

In female rats, five sarcomas, NOS, of the peritoneum were found in the high-dose group, but none were observed in the other groups studied. The result of the Cochran-Armitage test on the incidence of this tumor is significant (P = 0.010), using the mid- and high-dose vehicle-control group, the mid-dose group, and the high-dose group, but the results of the Fisher exact test are not significant. Results of statistical tests on the incidences of this tumor in male rats are not significant.

When sarcoma and other related tumors of the peritoneum are considered together, the results of the statistical tests are significant in each sex. The results of the Cochran-Armitage test, using the mid- and high-dose vehicle-control group, the mid-dose group, and the high-dose group indicate probability levels of P = 0.006 in males and P = 0.002 in females, and the Fisher exact comparisons of the incidences in the high-dose groups with those in the control groups are P = 0.033 in males and P = 0.016 in females; however, the P value for the males is above the 0.025 level required for significance by the multiple comparison criterion. The statistical conclusion is that the incidence of these tumors is dose associated in female rats.

In summary, the statistical tests indicate dose association in the incidence of osteosarcoma of the musculoskeletal system and in osteosarcoma at all sites in male rats, and also in sarcoma and other related tumors of the peritoneum in female rats.

IV. RESULTS - MICE

A. Body Weights and Clinical Signs (Mice)

Mean body weights of the high-, upper mid-, and lower mid-dose male mice and of all treated female groups were generally lower than those of the untreated- and vehicle-control groups (figures 6 and 7), while the weights of the low-dose males were more comparable to those of the control groups. Fluctuations in the growth curve may be due to mortality; as the size of a group diminishes, the mean body weight may be subject to wide variation.

Abdominal distention was the only consistent clinical sign reported in the treated animals; it occurred in all but the high-dose group, in which the time of survival was very short. To control respiratory disease, propylene glycol vapor was used during weeks 11 to 22 in the room housing the low-dose mice.

B. Survival (Mice)

The Kaplan and Meier curves estimating the probabilities of survival for male and female mice administered acronycine by injection at the doses of this experiment, together with the untreated and vehicle controls, are shown in figures 8 and 9.

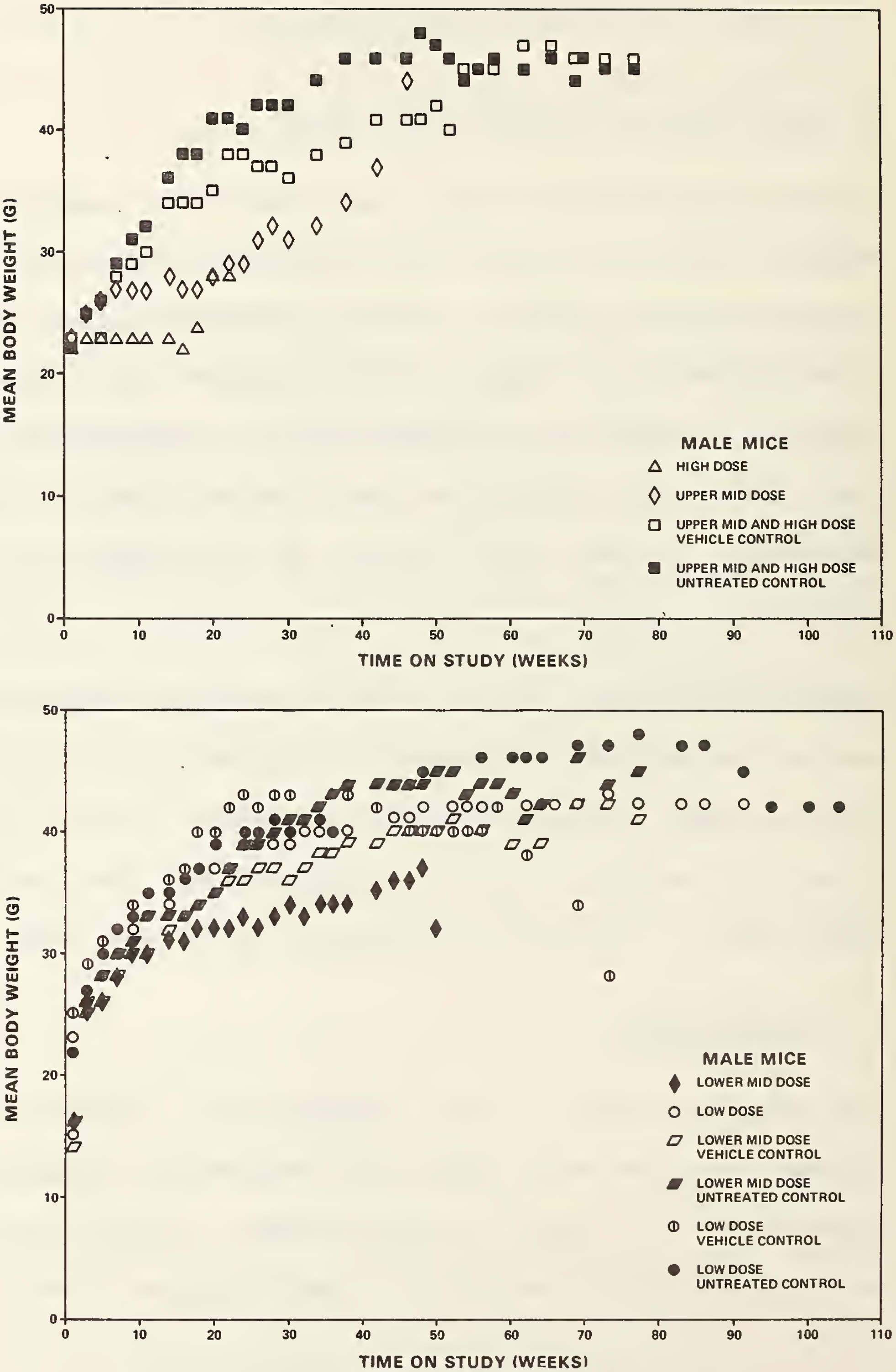


Figure 6. Growth Curves for Male Mice Treated with Acronycine

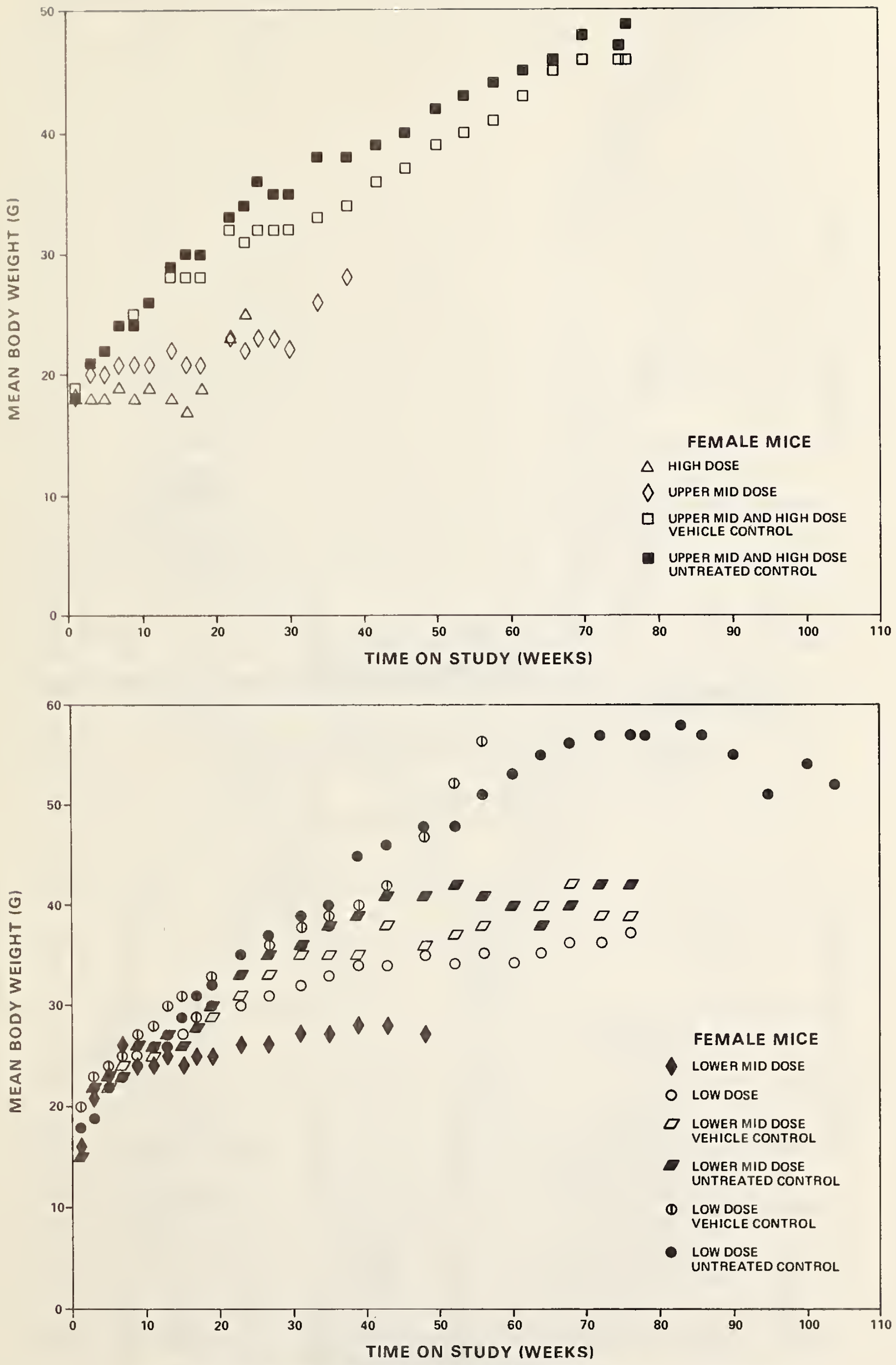


Figure 7. Growth Curves for Female Mice Treated with Acronycine

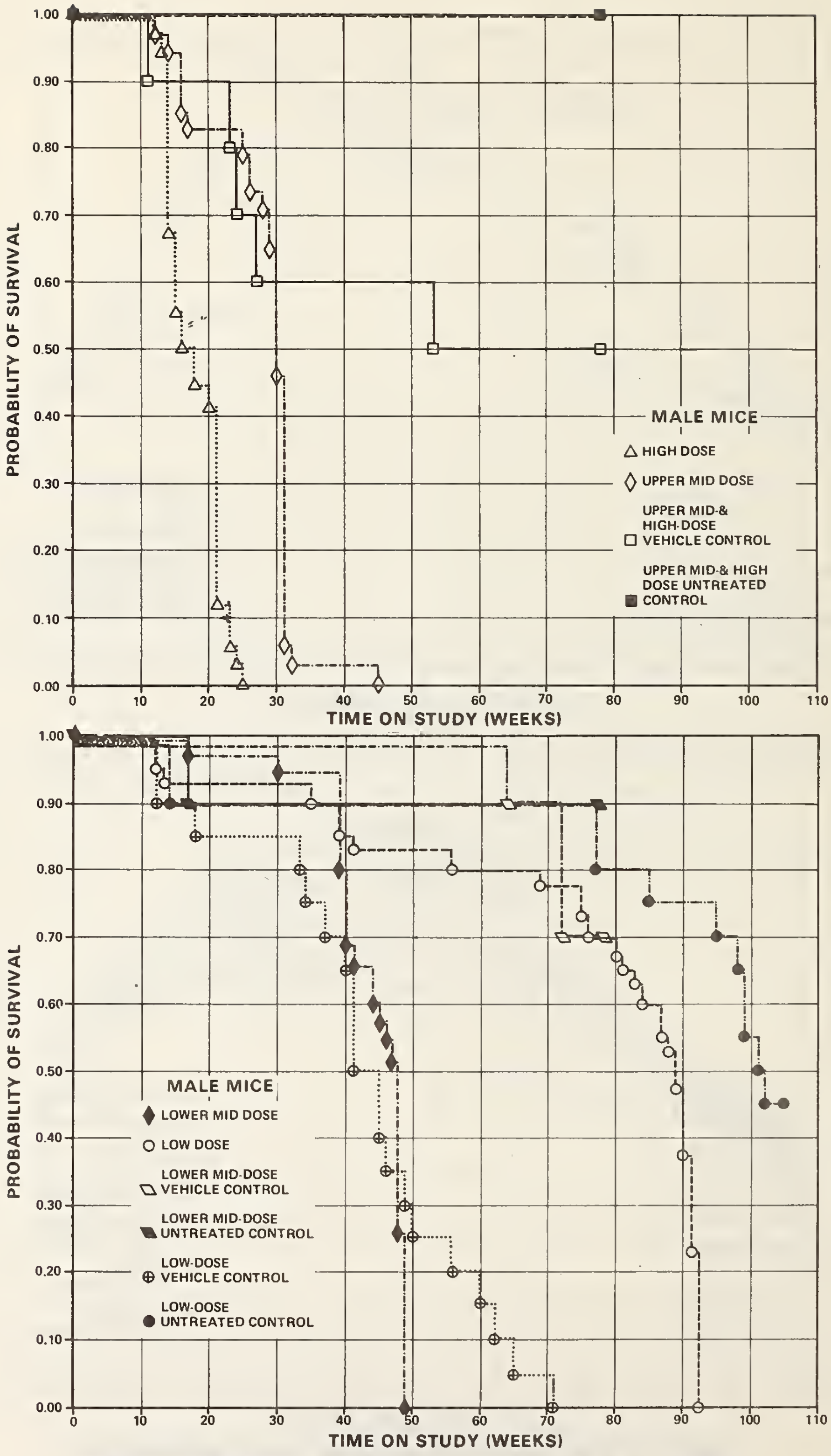


Figure 8. Survival Curves for Male Mice Treated with Acronycine

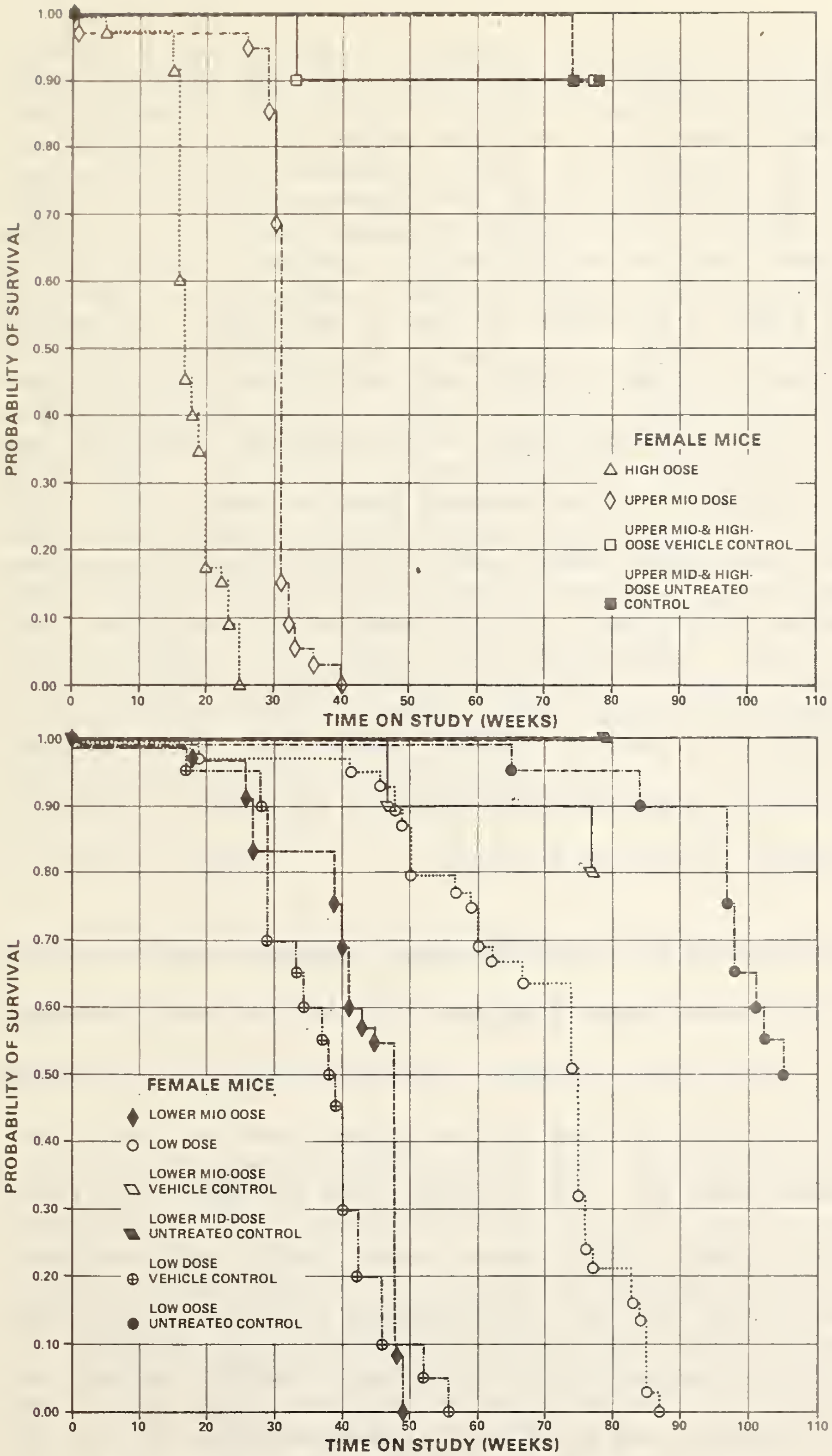


Figure 9. Survival Curves for Female Mice Treated with Acronycine

In each sex, the result of the Tarone test for positive dose-related trend in mortality over the period of the bioassay is significant ($P < 0.001$), using the high-dose group, the upper mid-dose group, and the vehicle-control groups; all animals in the treated groups died before the end of the study. The median number of weeks on study of male mice was 18 for the high-dose group, 30 for the upper mid-dose, 48 for the lower mid-dose, and 89 for the low-dose. In the low-dose group of male mice, 33/40 (82%) animals were alive after week 52 on study, and no tumor was observed before this time. In the lower mid-, upper mid-, and high-dose groups, all 35 male mice in each group died before week 52. No tumor was observed in the lower mid- and high-dose groups, but in the upper mid-dose group, a carcinoma of the bile duct was observed at week 30 on study.

In females, the median number of weeks on study was 17 for the high-dose, 31 for the upper mid-dose, 48 for the lower mid-dose and 74 for the low-dose groups. In the low-dose group, 31/40 (78%) animals lived to week 52 on study, and no tumor was observed before week 52. All 35 female mice in each of the three other treated groups (lower mid-, upper mid-, and high-dose groups) died before week 52. No tumor was observed in the lower mid- and high-dose groups, while in the upper mid-dose group, two tumors were observed, one at week 29 (adenocarcinoma, NOS, of the bile duct) and the other at week 32 (granulocytic leukemia of the bone marrow). The survival rates of the control groups within each sex are not comparable, since, in male mice, the percentage survivals to 78 weeks among the upper mid- and high-dose, lower mid-dose, and low-dose vehicle-control groups are 5/10 (50%), 7/10 (70%), and 0/20 (0%), respectively; among the corresponding untreated-control groups, they are 10/10 (100%), 9/10 (90%), and 16/20 (80%). In females, the percentage survivals to 78 weeks among the three vehicle-control groups are 9/10 (90%), 8/10 (80%), and 0/20 (0%); among the untreated-control groups, they are 9/10 (90%), 10/10 (100%), and 19/20 (95%). The early deaths of the treated mice of both sexes may have suppressed the incidences of late-appearing tumors.

C. Pathology (Mice)

Histopathologic findings on neoplasms in mice are summarized in Appendix B, tables B1-B4; findings on nonneoplastic lesions are summarized in Appendix D, tables D1-D4.

A variety of neoplasms were observed at approximately the same incidence in the control mice as in the low-dose mice. No neoplasms were observed in any of the high-dose mice, and very few neoplasms were observed in upper and lower mid-dose mice. There was a high incidence of early deaths and killed moribund animals in these three treated groups of animals during the exposure period, which may be related to the unusually low incidence of neoplasia observed in these groups.

There were cases in this study in which some types of neoplasms occurred only in treated mice. These have been observed as spontaneously occurring neoplasms in this strain of mouse. The nature and low incidence of these neoplasms in this study provide no evidence that they are related to the administration of acronycine.

A variety of nonneoplastic lesions were observed in both control and treated mice. The only apparent acronycine-induced lesions observed in this study were acute and chronic inflammatory lesions involving the thoracic and abdominal viscera, renal medullary necrosis, and bile duct hyperplasia in several mice.

In the judgment of the pathologists, the results of this microscopic examination of mice receiving acronycine at any of the four doses are inconclusive. Although there were no obvious acronycine-induced neoplasms observed in the treated animals when compared with control animals, the high incidence of early deaths and killed animals in the treated groups precludes a definitive conclusion on the effect of acronycine in mice in this study.

D. Statistical Analyses of Results (Mice)

Tables F1 and F2 in Appendix F contain the time-adjusted statistical analyses of the incidences of those primary tumors that were observed in at least two animals in one group and with an incidence of at least 5% in one or more than one group. The untreated controls are not included in the tables and the analyses, since the test conditions of the vehicle controls more closely resembled those of the treated animals. This bioassay originally started with 25 mg/kg as the high dose. In both sexes, survival was low, and no tumors were observed in the high-dose groups. In the groups of male and female mice receiving 12.5 mg/kg (upper mid-dose groups) survival was also low, and only one tumor, a carcinoma of the bile duct, was observed among the male mice. In the upper mid-dose females, one animal had leukemia and another had adenocarcinoma of the bile duct. Subsequently, two other groups were started at doses of 6 mg/kg (lower mid-dose group) and 2 mg/kg (low-dose group). No tumors were observed in the lower mid-dose group. Since the survival and numbers of tumors observed in all groups except for the low-dose group and its control group were so low that meaningful analysis was precluded, only the low-dose group and its control group were subjected to statistical analysis. A summary of all tumors in all treated groups is given in tables B1-B4 of Appendix B.

No significant increase in incidences of tumors in the treated groups was observed when compared with their control groups, although statistical analysis of the incidence of tumors in the mice was performed using all mice evaluated histopathologically and also using only those animals that lived beyond week 52 or beyond the week of the first observation of a specific tumor, whichever number of weeks was smaller. In each sex, the incidences of lymphoma in the low-dose groups were lower than those observed in the respective controls. When the incidences of lymphoma in the untreated-control groups are compared with those of the corresponding vehicle-control groups, no significant difference is observed between the lower mid-dose vehicle controls (0/10 in each sex) and the lower mid-dose untreated controls (0/9 in males and 0/10 in females); however, a significant difference is observed between the low-dose vehicle controls (13/17 in males and 19/19 in females) and the low-dose untreated controls (3/18 in males and 6/19 in females). These extremely high incidences in the vehicle-control groups compared with the untreated groups may indicate procedural difficulties. Overall, the shortened life spans of the treated and vehicle-control groups of mice precluded meaningful evaluation.

V. DISCUSSION

Acronycine was toxic to both sexes of rats and mice when administered by intraperitoneal injection at the doses used in this bioassay. This is shown by the high mortality rates in all but the low-dose groups, and by the lower mean body weights in dosed rats and mice at all doses throughout most of the study. Because of this high number of deaths, time-adjusted statistics were used for the analyses of all incidences of tumors.

In male rats, the dose-related trend in the mid- and high-dose groups for the incidence of osteosarcoma of all sites was significant (P = 0.002) using the respective vehicle-control group (vehicle controls 0/8, mid-dose 13/30, high-dose 12/18). Comparisons of the individual groups with respective control groups were also significant for the mid-dose (P = 0.022) and high-dose (P = 0.002) groups, but not for the low-dose group. Most of these neoplasms were observed grossly as enlargements of the long bones of the limbs, but occasionally, the tumors appeared to involve only soft tissues, and primary bone involvement was not observed. In female rats, osteosarcoma was observed only in 1/8 high-dose animals.

Sarcomas and other related tumors of the peritoneum (listed in the appendixes as sarcoma, NOS; mesothelioma, NOS; malignant mesothelioma; and fibrosarcoma of the peritoneum or multiple organs) were observed in all three dosed groups of both male and female rats, but in none of the control groups (males: low-dose 5/30, mid-dose 3/26, high-dose 7/16; females: low-dose 1/35, mid-dose 5/30, high-dose 13/28). In both sexes, the dose-related trends were significant (males, P = 0.006; females, P = 0.002), and the comparison of the incidences in the high-dose females with the vehicle-control group was significant (P = 0.016). None of the incidences in the individual dosed groups of males were significant when compared with vehicle controls. However, since the tumors occurred in all dosed groups but did not occur in any of the historical-control animals at this laboratory, they are considered to be related to administration of the chemical.

In female rats, the incidence of all tumors of epithelial origin of the mammary gland was significant only at the low dose (low-dose vehicle controls 1/10, low-dose 22/35, P = 0.004). Adenocarcinomas of the mammary gland were observed in seven low-dose, five mid-dose, and two high-dose female rats, but in no control females. The reverse dose relationship of both benign and malignant tumors was probably due to the higher number of early deaths which occurred in the high-dose group.

All mice of each sex of the three upper dosed groups had died by week 52. Among the low-dose mice, 33/40 males and 31/40 females lived to week 52 on study; however, only 5/20 male and 1/20 female low-dose vehicle controls lived beyond 1 year. Among the high-, upper, and lower mid-dose groups, only one tumor was observed in males and two in females in the upper mid dose. Even among the low-dose groups, no tumor was observed in a statistically significant incidence.

Lymphomas were observed at lower incidences in the low-dose male mice (10/37) and low-dose females (6/37) than in the corresponding male (13/17) and female (19/19) low-dose vehicle controls. However, the incidences in the upper mid-dose and high-dose vehicle-control and the lower mid-dose vehicle-control groups were not increased. When the incidences of lymphoma in the untreated- and vehicle-control groups were compared, no significant differences were observed between the lower mid-dose vehicle controls (0/10 in both sexes) and the lower mid-dose untreated controls (0/9 in males and 0/10 in females); however, a significant difference was observed between the low-dose vehicle controls (13/17 in males and 19/19 in females) and the low-dose untreated controls (3/18 in males and 6/19 in females).

This high incidence in the low-dose vehicle controls may have been due to a procedural problem. The same needle for injection was used for each group of five animals within a cage, and furthermore, the same bottle of vehicle solution was used for all vehicle-control animals. Thus, the possibility of transfer of tumor cells or oncogenic viruses cannot be excluded.

Nonneoplastic lesions of the peritoneal cavity, i.e., inflammation and fibrosis, were found in rats and mice from each of the dosed groups, but not in any control animals.

Since 1966, acronycine has been tested as an antineoplastic agent in humans; however, no long-term studies in animals or humans have been reported. In a 6-month study for pulmonary tumor response in strain A mice, Stoner et al. (1973) found that intraperitoneal injection of total doses of 0.53 to 2.60 mg/kg of acronycine did not elicit a carcinogenic response.

The vehicle used for the acronycine for all groups in this bioassay contained polysorbate 80, which in itself has been implicated as a carcinogen, but only in the production of local sarcomas following subcutaneous injections (Grasso et al., 1971). However, in these bioassays no local sarcomas were observed in the vehicle-control animals administered polysorbate 80 by intraperitoneal injection.

It is concluded that under the conditions of this bioassay, the low survival of the dosed and control mice and the possible procedural problems associated with intraperitoneal injection of the chemical do not allow a determination to be made of the carcinogenicity of acronycine in this species. In Sprague-Dawley rats, acronycine in the vehicle of 0.05% polysorbate 80 in phosphate-buffered saline was carcinogenic, producing tumors of the mammary gland in females, osteosarcomas in males, and sarcomas and other related tumors of the peritoneum in both males and females.

VI. BIBLIOGRAPHY

Armitage, P., Statistical Methods in Medical Research, John Wiley & Sons, Inc., New York, 1971, pp. 362-365.

Berenblum, I., ed., Carcinogenicity Testing: A Report of the Panel on Carcinogenicity of the Cancer Research Commission of the UICC, Vol. 2. International Union Against Cancer, Geneva, 1969.

Carter, S. K., Clinical trials and combination chemotherapy. Cancer Chemotherp. Reports Part III 2(1):81-97, 1971.

Cox, D. R., Regression models and life tables. J. R. Statist. Soc. B 34:187-220, 1972.

Cox, D. R., Analysis of Binary Data, Methuen & Co., Ltd. London, 1970, pp. 48-52.

Dunn, B. P., Gout, P. W., and Beer, C. T., Effects of the antineoplastic alkaloid acronycine on nucleoside uptake and incorporation into nucleic acids by cultured L5178Y cells. Cancer Res. 33:2310-2319, 1973.

Gart, J. J., The comparison of proportions: a review of significance tests, confidence limits and adjustments for stratification. Rev. Int. Stat. Inst. 39:148-169, 1971.

Grasso, P., Gangolli, S. D., Goldberg, L., and Hooson, J., Physicochemical and other factors determining local sarcoma production by food additives. Fd. Cosmet. Toxicol. 9:463-478, 1971.

Kaplan, E. L. and Meier, P., Nonparametric estimation from incomplete observations. J. Am. Statist. Assoc. 53:457-481, 1958.

Lahey, F. N. and Thomas, W. C., Alkaloids of the Australian Rutaceae: Acronychia Baueri. Australian J. Sci. Res. 2A:423, 1949.

Linhart, M. S., Cooper, J. A., Martin, R. L., Page, N. P., and Peters, J. A., Carcinogenesis bioassay data system. Comp. and Biomed. Res. 7:230-248, 1974.

Miller, R. G., Jr., Simultaneous Statistical Inference, McGraw-Hill Book Co., New York, 1966, pp. 6-10.

Saffiotti, U., Montesano, R., Sellakumar, A. R., Cefis, F., and Kaufman, D. G., Respiratory tract carcinogenesis in hamsters induced by different numbers of administrations of benzo(a) pyrene and ferric oxide. Cancer Res. 32:1073-1081, 1972.

Stoner, G. D., Shimkin, M. B., Kniazeff, A. J., Weisburger, J. H. Weisburger, E. K., and Gori, G. B., Test for carcinogenicity of food additives and chemotherapeutic agents by the pulmonary tumor response in strain A mice. Cancer Res. 33:3069-3085, 1973.

Svoboda, G. H., Poore, G. A., Simpson, P. J., and Boder, G. B., Alkaloids of Acronychia Baueri Schott I. J. Pharm. Sci. 55:758-768, 1966.

Tarone, R. E. Tests for trend in life table analysis. Biometrika 62:679-682, 1975.

APPENDIX A

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN RATS GIVEN INTRAPERITONEAL INJECTIONS OF ACRONYCINE

TABLE A1

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS GIVEN INTRAPERITONEAL INJECTIONS OF ACRONYCINE (CONTROL GROUPS)

	LOW DOSE UNTREATED CONTROL	MID AND HIGH DOSE UNTREATED CONTROL	LOW DOSE VEHICLE CONTROL	MID AND HIGH DOSE VEHICLE CONTROL
ANIMALS INITIALLY IN STUDY	10	10	10	10
ANIMALS NECROPSIED	10	9	10	8
ANIMALS EXAMINED HISTOPATHOLOGICALLY	10	9	10	8
INTEGUMENTARY SYSTEM				
NONE				
RESPIRATORY SYSTEM				
#LUNG	(10)	(9)	(10)	(8)
ALVEOLAR/BRONCHIOLAR ADENOMA				1 (13%)
HEMATOPOIETIC SYSTEM				
NONE				
CIRCULATORY SYSTEM				
NONE				
DIGESTIVE SYSTEM				
NONE				
URINARY SYSTEM				
NONE				
ENDOCRINE SYSTEM				
#PITUITARY	(5)	(8)	(4)	(7)
CHROMOPHOBE ADENOMA				2 (29%)
CHROMOPHOBE CARCINOMA		1 (13%)		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A1 CONTROL MALE RATS: NEOPLASMS (CONTINUED)

	LOW DOSE UNTREATED CONTROL	MID AND HIGH DOSE UNTREATED CONTROL	LOW DOSE VEHICLE CONTROL	MID AND HIGH DOSE VEHICLE CONTROL
ACIDOPHIL ADENOMA				1 (14%)
#ADRENAL	(10)	(9)	(10)	(8)
CORTICAL ADENOMA				1 (13%)
REPRODUCTIVE SYSTEM				
*MAMMARY GLAND	(10)	(9)	(10)	(8)
ADENOCARCINOMA, NOS				1 (13%)
NERVOUS SYSTEM				
NONE				
SPECIAL SENSE ORGANS				
NONE				
MUSCULOSKELETAL SYSTEM				
NONE				
BODY CAVITIES				
NONE				
ALL OTHER SYSTEMS				
NONE				
ANIMAL DISPOSITION SUMMARY				
ANIMALS INITIALLY IN STUDY	10	10	10	10
NATURAL DEATH@		3		2
MORIBUND SACRIFICE				1
SCHEDULED SACRIFICE				
ACCIDENTALLY KILLED				
TERMINAL SACRIFICE	10	7	10	7
ANIMAL MISSING				

@ INCLUDES AUTOLYZED ANIMALS

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A1 CONTROL MALE RATS: NEOPLASMS (CONTINUED)

	LOW DOSE UNTREATED CONTROL	MID AND HIGH DOSE UNTREATED CONTROL	LOW DOSE VEHICLE CONTROL	MID AND HIGH DOSE VEHICLE CONTROL
TUMOR SUMMARY				
TOTAL ANIMALS WITH PRIMARY TUMORS*		1		4
TOTAL PRIMARY TUMORS		1		6
TOTAL ANIMALS WITH BENIGN TUMORS				4
TOTAL BENIGN TUMORS				5
TOTAL ANIMALS WITH MALIGNANT TUMORS		1		1
TOTAL MALIGNANT TUMORS		1		1
TOTAL ANIMALS WITH SECONDARY TUMORS#				
TOTAL SECONDARY TUMORS				
TOTAL ANIMALS WITH TUMORS UNCERTAIN- BENIGN OR MALIGNANT				
TOTAL UNCERTAIN TUMORS				
TOTAL ANIMALS WITH TUMORS UNCERTAIN- PRIMARY OR METASTATIC				
TOTAL UNCERTAIN TUMORS				

* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS

# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN

TABLE A2

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS GIVEN INTRAPERITONEAL INJECTIONS OF ACRONYCINE (TREATED GROUPS)

	LOW DOSE	MID DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	35	35	35
ANIMALS NECROPSIED	31	32	34
ANIMALS EXAMINED HISTOPATHOLOGICALLY	30	31	34
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(31)	(32)	(34)
SARCOMA, NOS	1 (3%)		
FIBROMA	1 (3%)	1 (3%)	
FIBROSARCOMA			1 (3%)
OSTEOSARCOMA		1 (3%)	1 (3%)
RESPIRATORY SYSTEM			
#LUNG	(30)	(31)	(34)
ALVEOLAR/BRONCHIOLAR ADENOMA	1 (3%)		
HEMANGIOSARCOMA, METASTATIC	1 (3%)		
OSTEOSARCOMA, METASTATIC	2 (7%)	9 (29%)	10 (29%)
HEMATOPOIETIC SYSTEM			
#LYMPH NODE	(30)	(20)	(21)
OSTEOSARCOMA, METASTATIC		1 (5%)	1 (5%)
#MESENTERIC L. NODE	(30)	(20)	(21)
MESOTHELIOMA, METASTATIC	1 (3%)		
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(29)	(31)	(34)
HEPATOCELLULAR ADENOMA	1 (3%)		
HEPATOCELLULAR CARCINOMA	1 (3%)		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A2 TREATED MALE RATS: NEOPLASMS (CONTINUED)

	LOW DOSE	MID DOSE	HIGH DOSE
FIBROSARCOMA			1 (3%)
HEMANGIOSARCOMA	4 (14%)		1 (3%)
OSTEOSARCOMA			2 (6%)
OSTEOSARCOMA, METASTATIC	3 (10%)	4 (13%)	5 (15%)
*BILE DUCT	(31)	(32)	(34)
BILE DUCT CARCINOMA	2 (6%)		
#PANCREAS	(24)	(26)	(30)
FIBROSARCOMA		1 (4%)	
OSTEOSARCOMA, METASTATIC		1 (4%)	1 (3%)
#STOMACH	(29)	(30)	(30)
OSTEOSARCOMA, METASTATIC		1 (3%)	
#LARGE INTESTINE	(24)	(29)	(28)
SQUAMOUS CELL CARCINOMA		1 (3%)	
URINARY SYSTEM			
#KIDNEY	(29)	(31)	(33)
OSTEOSARCOMA, METASTATIC		1 (3%)	1 (3%)
ENDOCRINE SYSTEM			
#ADRENAL	(28)	(31)	(33)
CORTICAL ADENOMA	1 (4%)	2 (6%)	4 (12%)
CORTICAL CARCINOMA		1 (3%)	
OSTEOSARCOMA, METASTATIC	1 (4%)		3 (9%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(31)	(32)	(34)
FIBROADENOMA		1 (3%)	
#TESTIS	(28)	(30)	(32)
INTERSTITIAL-CELL TUMOR	1 (4%)		
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY

* NUMBER OF ANIMALS NECROPSIED

TABLE A2 TREATED MALE RATS: NEOPLASMS (CONTINUED)

	LOW DOSE	MID DOSE	HIGH DOSE
MUSCULOSKELETAL SYSTEM			
*BONE	(31)	(32)	(34)
OSTEOSARCOMA	3 (10%)	10 (31%)	8 (24%)
*VERTEBRA	(31)	(32)	(34)
OSTEOSARCOMA		1 (3%)	1 (3%)
*SKELETAL MUSCLE	(31)	(32)	(34)
OSTEOSARCOMA, METASTATIC			1 (3%)
BODY CAVITIES			
*ABDOMINAL CAVITY	(31)	(32)	(34)
FIBROSARCOMA			1 (3%)
*PERITONEUM	(31)	(32)	(34)
SARCOMA, NOS	2 (6%)		1 (3%)
FIBROSARCOMA			2 (6%)
MESOTHELIOMA, NOS	1 (3%)	1 (3%)	
MESOTHELIOMA, MALIGNANT	2 (6%)		
HEMANGIOSARCOMA, METASTATIC	1 (3%)		
OSTEOSARCOMA			1 (3%)
OSTEOSARCOMA, METASTATIC	1 (3%)		
*PERITONEAL CAVITY	(31)	(32)	(34)
FIBROSARCOMA			1 (3%)
*MESENTERY	(31)	(32)	(34)
OSTEOSARCOMA		1 (3%)	
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(31)	(32)	(34)
SARCOMA, NOS, METASTATIC	2 (6%)		
FIBROSARCOMA		2 (6%)	2 (6%)
OSTEOSARCOMA, METASTATIC	1 (3%)	2 (6%)	
DIAPHRAGM			
FIBROSARCOMA			1

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A2 TREATED MALE RATS: NEOPLASMS (CONTINUED)

	LOW DOSE	MID DOSE	HIGH DOSE
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	35	35	35
NATURAL DEATH@	17	17	9
MORIBUND SACRIFICE	9	17	26
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	9	1	
ANIMAL MISSING			

@ INCLUDES AUTOLYZED ANIMALS

	LOW DOSE	MID DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	17	16	15
TOTAL PRIMARY TUMORS	21	23	28
TOTAL ANIMALS WITH BENIGN TUMORS	5	4	4
TOTAL BENIGN TUMORS	5	4	4
TOTAL ANIMALS WITH MALIGNANT TUMORS	12	16	14
TOTAL MALIGNANT TUMORS	15	18	24
TOTAL ANIMALS WITH SECONDARY TUMORS#	8	12	10
TOTAL SECONDARY TUMORS	13	19	22
TOTAL ANIMALS WITH TUMORS UNCERTAIN- BENIGN OR MALIGNANT	1	1	
TOTAL UNCERTAIN TUMORS	1	1	
TOTAL ANIMALS WITH TUMORS UNCERTAIN- PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS
# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN

TABLE A3

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS GIVEN INTRAPERITONEAL INJECTIONS OF ACRONYCINE (CONTROL GROUPS)

	LOW DOSE UNTREATED CONTROL	MID & HIGH DOSE UNTREATED CONTROL	LOW DOSE VEHICLE CONTROL	MID & HIGH DOSE VEHICLE CONTROL
ANIMALS INITIALLY IN STUDY	10	10	10	10
ANIMALS NECROPSIED	10	9	10	9
ANIMALS EXAMINED HISTOPATHOLOGICALLY	10	8	10	9
INTEGUMENTARY SYSTEM				
NONE				
RESPIRATORY SYSTEM				
#TRACHEA	(10)	(8)	(10)	(9)
CARCINOMA, NOS, METASTATIC				1 (11%)
#LUNG	(10)	(7)	(10)	(9)
ALVEOLAR/BRONCHIOLAR CARCINOMA				1 (11%)
HEMATOPOIETIC SYSTEM				
NONE				
CIRCULATORY SYSTEM				
NONE				
DIGESTIVE SYSTEM				
#ESOPHAGUS	(10)	(8)	(10)	(6)
CARCINOMA, NOS, METASTATIC				1 (17%)
URINARY SYSTEM				
NONE				
ENDOCRINE SYSTEM				
#PITUITARY	(7)	(7)	(6)	(9)
CHROMOPHOBE ADENOMA	3 (43%)	3 (43%)	2 (33%)	1 (11%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A3 CONTROL FEMALE RATS: NEOPLASMS (CONTINUED)

	LOW DOSE UNTREATED CONTROL	MID & HIGH DOSE UNTREATED CONTROL	LOW DOSE VEHICLE CONTROL	MID & HIGH DOSE VEHICLE CONTROL
CHROMOPHOBE CARCINOMA		1 (14%)		
#ADRENAL	(10)	(8)	(10)	(9)
CORTICAL ADENOMA				1 (11%)
#THYROID	(10)	(7)	(10)	(7)
CARCINOMA,NOS				1 (14%)
REPRODUCTIVE SYSTEM				
*MAMMARY GLAND	(10)	(9)	(10)	(9)
FIBROADENOMA	4 (40%)	1 (11%)	1 (10%)	3 (33%)
#CERVIX UTERI	(10)	(8)	(10)	(9)
SQUAMOUS CELL PAPILLOMA			1 (10%)	
NERVOUS SYSTEM				
NONE				
SPECIAL SENSE ORGANS				
NONE				
MUSCULOSKELETAL SYSTEM				
NONE				
BODY CAVITIES				
NONE				
ALL OTHER SYSTEMS				
NONE				

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A3 CONTROL FEMALE RATS: NEOPLASMS (CONTINUED)

	LOW DOSE UNTREATED CONTROL	MID & HIGH DOSE UNTREATED CONTROL	LOW DOSE VEHICLE CONTROL	MID & HIGH DOSE VEHICLE CONTROL
ANIMAL DISPOSITION SUMMARY				
ANIMALS INITIALLY IN STUDY	10	10	10	10
NATURAL DEATH@	1	1	1	2
MORIBUND SACRIFICE	1	1		
SCHEDULED SACRIFICE				
ACCIDENTALLY KILLED				
TERMINAL SACRIFICE	8	8	9	8
ANIMAL MISSING				

@ INCLUDES AUTOLYZED ANIMALS

	LOW DOSE UNTREATED CONTROL	MID & HIGH DOSE UNTREATED CONTROL	LOW DOSE VEHICLE CONTROL	MID & HIGH DOSE VEHICLE CONTROL
TUMOR SUMMARY				
TOTAL ANIMALS WITH PRIMARY TUMORS*	5	4	4	3
TOTAL PRIMARY TUMORS	7	5	4	7
TOTAL ANIMALS WITH BENIGN TUMORS	5	3	4	3
TOTAL BENIGN TUMORS	7	4	4	5
TOTAL ANIMALS WITH MALIGNANT TUMORS		1		2
TOTAL MALIGNANT TUMORS		1		2
TOTAL ANIMALS WITH SECONDARY TUMORS#				1
TOTAL SECONDARY TUMORS				2
TOTAL ANIMALS WITH TUMORS UNCERTAIN- BENIGN OR MALIGNANT				
TOTAL UNCERTAIN TUMORS				
TOTAL ANIMALS WITH TUMORS UNCERTAIN- PRIMARY OR METASTATIC				
TOTAL UNCERTAIN TUMORS				

* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS

SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN

TABLE A4

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS GIVEN INTRAPERITONEAL INJECTIONS OF ACRONYCINE (TREATED GROUPS)

	LOW DOSE	MID DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	35	35	35
ANIMALS NECROPSIED	35	32	34
ANIMALS EXAMINED HISTOPATHOLOGICALLY	35	32	33
INTEGUMENTARY SYSTEM			
*SKIN	(35)	(32)	(34)
FIBROSARCOMA		1 (3%)	
*SUBCUT TISSUE	(35)	(32)	(34)
CARCINOMA,NOS			1 (3%)
SARCOMA, NOS	4 (11%)		
FIBROMA	2 (6%)		1 (3%)
FIBROSARCOMA	1 (3%)	2 (6%)	
LIPOMA	1 (3%)		
RHABDOMYOSARCOMA			1 (3%)
RESPIRATORY SYSTEM			
#LUNG	(35)	(32)	(33)
CARCINOMA, NOS, METASTATIC			4 (12%)
ALVEOLAR/BRONCHIOLAR ADENOMA		2 (6%)	
ALVEOLAR/BRONCHIOLAR CARCINOMA	1 (3%)		
SARCOMA, NOS			1 (3%)
SARCOMA, NOS, METASTATIC			1 (3%)
HEMANGIOSARCOMA, METASTATIC	1 (3%)		1 (3%)
HEMATOPOIETIC SYSTEM			
#LYMPH NODE	(35)	(12)	(28)
CARCINOMA, NOS, METASTATIC			1 (4%)
#THYMUS	(14)	(16)	(6)
CARCINOMA, NOS, METASTATIC			1 (17%)
CIRCULATORY SYSTEM			
NONE			

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A4 TREATED FEMALE RATS: NEOPLASMS (CONTINUED)

	LOW DOSE	MID DOSE	HIGH DOSE
DIGESTIVE SYSTEM			
#LIVER	(35)	(32)	(33)
CARCINOMA, NOS, METASTATIC			1 (3%)
HEPATOCELLULAR ADENOMA		4 (13%)	
HEPATOCELLULAR CARCINOMA		1 (3%)	
HEMANGIOSARCOMA	5 (14%)		1 (3%)
OSTEOSARCOMA, METASTATIC			1 (3%)
#PANCREAS	(34)	(30)	(31)
CARCINOMA,NOS			1 (3%)
SARCOMA, NOS			1 (3%)
OSTEOSARCOMA, METASTATIC			1 (3%)
#STOMACH	(35)	(30)	(32)
SARCOMA, NOS			1 (3%)
OSTEOSARCOMA, METASTATIC			1 (3%)
#ILEUM	(35)	(29)	(30)
FIBROSARCOMA			1 (3%)
#LARGE INTESTINE	(35)	(29)	(31)
SARCOMA, NOS			2 (6%)
URINARY SYSTEM			
#KIDNEY	(35)	(31)	(32)
FIBROMA			1 (3%)
#URINARY BLADDER	(32)	(26)	(31)
PAPILLOMA, NOS		1 (4%)	
OSTEOSARCOMA, METASTATIC			1 (3%)
ENDOCRINE SYSTEM			
#PITUITARY	(28)	(30)	(30)
CHROMOPHOBE ADENOMA	4 (14%)	1 (3%)	1 (3%)
#ADRENAL	(32)	(30)	(31)
CORTICAL ADENOMA		9 (30%)	7 (23%)
CORTICAL CARCINOMA			1 (3%)
#ADRENAL CORTEX	(32)	(30)	(31)
CORTICAL ADENOMA		1 (3%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A4 TREATED FEMALE RATS: NEOPLASMS (CONTINUED)

	LOW DOSE	MID DOSE	HIGH DOSE
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(35)	(32)	(34)
ADENOCARCINOMA, NOS	6 (17%)	4 (13%)	2 (6%)
PAPILLARY ADENOCARCINOMA	1 (3%)		
CYSTADENOMA, NOS		1 (3%)	
CYSTADENOCARCINOMA, NOS		1 (3%)	
FIBROMA		2 (6%)	1 (3%)
FIBROADENOMA	20 (57%)	13 (41%)	3 (9%)
#UTERUS	(34)	(32)	(32)
SARCOMA, NOS			1 (3%)
LEIOMYOSARCOMA		1 (3%)	
ENDOMETRIAL STROMAL POLYP	5 (15%)		1 (3%)
#OVARY	(31)	(31)	(31)
LEIOMYOMA		1 (3%)	
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
*SKELETAL MUSCLE	(35)	(32)	(34)
SARCOMA, NOS			1 (3%)
BODY CAVITIES			
*ABDOMINAL CAVITY	(35)	(32)	(34)
FIBROSARCOMA		1 (3%)	1 (3%)
*PERITONEUM	(35)	(32)	(34)
SARCOMA, NOS			5 (15%)
FIBROSARCOMA		2 (6%)	
MESOTHELIOMA, MALIGNANT	1 (3%)		2 (6%)
OSTEOSARCOMA			1 (3%)

# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A4 TREATED FEMALE RATS: NEOPLASMS (CONTINUED)

	LOW DOSE	MID DOSE	HIGH DOSE
*MESENTERY	(35)	(32)	(34)
SARCOMA, NOS			1 (3%)
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(35)	(32)	(34)
SARCOMA, NOS		2 (6%)	3 (9%)
FIBROSARCOMA		2 (6%)	1 (3%)
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	35	35	35
NATURAL DEATH@	5	7	9
MORIBUND SACRIFICE	8	9	24
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	22	19	2
ANIMAL MISSING			

@ INCLUDES AUTOLYZED ANIMALS

	LOW DOSE	MID DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	31	20	25
TOTAL PRIMARY TUMORS	51	52	44
TOTAL ANIMALS WITH BENIGN TUMORS	24	18	10
TOTAL BENIGN TUMORS	32	35	15
TOTAL ANIMALS WITH MALIGNANT TUMORS	16	12	20
TOTAL MALIGNANT TUMORS	19	17	29
TOTAL ANIMALS WITH SECONDARY TUMORS#	1		6
TOTAL SECONDARY TUMORS	1		13
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT			
TOTAL UNCERTAIN TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS

# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN

APPENDIX B

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MICE GIVEN INTRAPERITONEAL INJECTIONS OF ACRONYCINE

TABLE B1

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE GIVEN INTRAPERITONEAL INJECTIONS OF ACRONYCINE (CONTROL GROUPS)

	LOWER MID DOSE UNTREATED CONTROL	UPPER MID AND HIGH DOSE UNTREATED CONTROL	LOW DOSE UNTREATED CONTROL	LOWER MID DOSE VEHICLE CONTROL	UPPER MID AND HIGH DOSE VEHICLE CONTROL
ANIMALS INITIALLY IN STUDY	10	10	20	10	10
ANIMALS NECROPSIED	9	10	20	10	10
ANIMALS EXAMINED HISTOPATHOLOGICALLY	9	10	20	10	10
INTEGUMENTARY SYSTEM					
*SKIN	(9)	(10)	(20)	(10)	(10)
PAPILLOMA, NOS			1 (5%)		
*SUBCUT TISSUE	(9)	(10)	(20)	(10)	(10)
SARCOMA, NOS			2 (10%)		
RESPIRATORY SYSTEM					
#LUNG	(9)	(10)	(20)	(10)	(10)
HEPATOCELLULAR CARCINOMA, METAST		1 (10%)			
ALVEOLAR/BRONCHIOLAR ADENOMA		1 (10%)	1 (5%)		
ALVEOLAR/BRONCHIOLAR CARCINOMA			2 (10%)		
HEMATOPOIETIC SYSTEM					
*MULTIPLE ORGANS	(9)	(10)	(20)	(10)	(10)
MALIG.LYMPHOMA, UNDIFFER-TYPE			3 (15%)		
LYMPHOCYTIC LEUKEMIA				3 (30%)	
#INGUINAL LYMPH NODE	(9)	(9)	(20)	(10)	(9)
SARCOMA, NOS, METASTATIC			1 (5%)		
CIRCULATORY SYSTEM					
NONE					
DIGESTIVE SYSTEM					
#LIVER	(9)	(10)	(20)	(10)	(10)
HEPATOCELLULAR ADENOMA		2 (20%)	4 (20%)	1 (10%)	
HEPATOCELLULAR CARCINOMA	1 (11%)	2 (20%)	3 (15%)	1 (10%)	

# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B1 CONTROL MALE MICE: NEOPLASMS (CONTINUED)

	LOWER MID DOSE UNTREATED CONTROL	UPPER MID AND HIGH DOSE UNTREATED CONTROL	LOW DOSE UNTREATED CONTROL	LOWER MID DOSE VEHICLE CONTROL	UPPER MID AND HIGH DOSE VEHICLE CONTROL
URINARY SYSTEM					
NONE					
ENDOCRINE SYSTEM					
NONE					
REPRODUCTIVE SYSTEM					
NONE					
NERVOUS SYSTEM					
NONE					
SPECIAL SENSE ORGANS					
NONE					
MUSCULOSKELETAL SYSTEM					
*FEMUR	(9)	(10)	(20)	(10)	(10)
OSTEOCHONDROMA			1 (5%)		
BODY CAVITIES					
*MESENTERY	(9)	(10)	(20)	(10)	(10)
LIPOMA					1 (10%)
ALL OTHER SYSTEMS					
NONE					

CONTINUED ON

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B1 CONTROL MALE MICE: NEOPLASMS (CONTINUED)

	LOWER MID DOSE UNTREATED CONTROL	UPPER MID AND HIGH DOSE UNTREATED CONTROL	LOW DOSE UNTREATED CONTROL	LOWER MID DOSE VEHICLE CONTROL	UPPER MID AND HIGH DOSE VEHICLE CONTROL
ANIMAL DISPOSITION SUMMARY					
ANIMALS INITIALLY IN STUDY	10	10	20	10	10
NATURAL DEATH@	1		5	2	5
MORIBUND SACRIFICE			6	1	
SCHEDULED SACRIFICE					
ACCIDENTALLY KILLED					
TERMINAL SACRIFICE	9	10	9	7	5
ANIMAL MISSING					

@ INCLUDES AUTOLYZED ANIMALS

	LOWER MID DOSE UNTREATED CONTROL	UPPER MID AND HIGH DOSE UNTREATED CONTROL	LOW DOSE UNTREATED CONTROL	LOWER MID DOSE VEHICLE CONTROL	UPPER MID AND HIGH DOSE VEHICLE CONTROL
TUMOR SUMMARY					
TOTAL ANIMALS WITH PRIMARY TUMORS*	1	4	13	5	1
TOTAL PRIMARY TUMORS	1	5	17	5	1
TOTAL ANIMALS WITH BENIGN TUMORS		3	6	1	1
TOTAL BENIGN TUMORS		3	7	1	1
TOTAL ANIMALS WITH MALIGNANT TUMORS	1	2	9	4	
TOTAL MALIGNANT TUMORS	1	2	10	4	
TOTAL ANIMALS WITH SECONDARY TUMORS#		1	1		
TOTAL SECONDARY TUMORS		1	1		
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT					
TOTAL UNCERTAIN TUMORS					
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC					
TOTAL UNCERTAIN TUMORS					

* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS
# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN

TABLE B2

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE GIVEN INTRAPERITONEAL INJECTIONS OF ACRONYCINE (CONTROL AND TREATED GROUPS)

	LOW DOSE VEHICLE CONTROL	LOW DOSE	LOWER MID DOSE	UPPER MID DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	40	35	35	35
ANIMALS MISSING				1	
ANIMALS NECROPSIED	20	40	35	34	29
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	40	35	33	12
INTEGUMENTARY SYSTEM					
NONE					
RESPIRATORY SYSTEM					
#LUNG	(18)	(40)	(35)	(33)	(12)
ALVEOLAR/BRONCHIOLAR ADENOMA		1 (3%)			
HEMATOPOIETIC SYSTEM					
*MULTIPLE ORGANS	(20)	(40)	(35)	(34)	(29)
MALIGNANT LYMPHOMA, NOS	2 (10%)				
MALIG.LYMPHOMA, UNDIFFER-TYPE	5 (25%)	6 (15%)			
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE	6 (30%)	3 (8%)			
#SPLEEN	(19)	(38)	(35)	(30)	(12)
HEMANGIOSARCOMA		1 (3%)			
#THYMUS	(5)	(7)	(15)	(2)	(9)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE		1 (14%)			
CIRCULATORY SYSTEM					
NONE					
DIGESTIVE SYSTEM					
*BILE DUCT	(20)	(40)	(35)	(34)	(29)
BILE DUCT CARCINOMA				1 (3%)	
URINARY SYSTEM					
NONE					

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B2 CONTROL & TREATED MALE MICE: NEOPLASMS (CONTINUED)

	LOW DOSE VEHICLE CONTROL	LOW DOSE	LOWER MID DOSE	UPPER MID DOSE	HIGH DOSE
ENDOCRINE SYSTEM					
#ADRENAL	(20)	(37)	(32)	(29)	(11)
PHEOCHROMOCYTOMA		1 (3%)			
#THYROID	(18)	(38)	(18)	(17)	(7)
FOLLICULAR-CELL CARCINOMA		1 (3%)			
REPRODUCTIVE SYSTEM					
#TESTIS	(20)	(38)	(34)	(31)	(12)
HEMANGIOSARCOMA		1 (3%)			
NERVOUS SYSTEM					
NONE					
SPECIAL SENSE ORGANS					
NONE					
MUSCULOSKELETAL SYSTEM					
*KNEE JOINT	(20)	(40)	(35)	(34)	(29)
OSTEOCHONDROMA		1 (3%)			
BODY CAVITIES					
NONE					
ALL OTHER SYSTEMS					
*MULTIPLE ORGANS	(20)	(40)	(35)	(34)	(29)
SARCOMA, NOS		2 (5%)			

CONTINUED

# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B2 CONTROL & TREATED MALE MICE: NEOPLASMS (CONTINUED)

	LOW DOSE VEHICLE CONTROL	LOW DOSE	LOWER MID DOSE	UPPER MID DOSE	HIGH DOSE
ANIMAL DISPOSITION SUMMARY					
ANIMALS INITIALLY IN STUDY	20	40	35	35	35
NATURAL DEATH@	8	12	12	19	25
MORIBUND SACRIFICE	12	28	23	14	9
SCHEDULED SACRIFICE					
ACCIDENTALLY KILLED				1	1
TERMINAL SACRIFICE					
ANIMAL MISSING				1	

@ INCLUDES AUTOLYZED ANIMALS

	LOW DOSE VEHICLE CONTROL	LOW DOSE	LOWER MID DOSE	UPPER MID DOSE	HIGH DOSE
TUMOR SUMMARY					
TOTAL ANIMALS WITH PRIMARY TUMORS*	13	14		1	
TOTAL PRIMARY TUMORS	13	18		1	
TOTAL ANIMALS WITH BENIGN TUMORS		3			
TOTAL BENIGN TUMORS		3			
TOTAL ANIMALS WITH MALIGNANT TUMORS	13	13		1	
TOTAL MALIGNANT TUMORS	13	15		1	
TOTAL ANIMALS WITH SECONDARY TUMORS#					
TOTAL SECONDARY TUMORS					
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT					
TOTAL UNCERTAIN TUMORS					
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC					
TOTAL UNCERTAIN TUMORS					

* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS

# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN

TABLE B3

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE GIVEN INTRAPERITONEAL INJECTIONS OF ACRONYCINE (CONTROL GROUPS)

	LOWER MID DOSE UNTREATED CONTROL	UPPER MID AND HIGH DOSE UNTREATED CONTROL	LOW DOSE UNTREATED CONTROL	LOWER MID DOSE VEHICLE CONTROL	UPPER MID AND HIGH DOSE VEHICLE CONTROL
ANIMALS INITIALLY IN STUDY	10	10	20	10	10
ANIMALS NECROPSIED	10	10	19	10	10
ANIMALS EXAMINED HISTOPATHOLOGICALLY	10	10	19	10	10
INTEGUMENTARY SYSTEM					
*SUBCUT TISSUE	(10)	(10)	(19)	(10)	(10)
SARCOMA, NOS			1 (5%)		
HEMANGIOMA			2 (11%)		
RESPIRATORY SYSTEM					
NONE					
HEMATOPOIETIC SYSTEM					
*MULTIPLE ORGANS	(10)	(10)	(19)	(10)	(10)
MALIG.LYMPHOMA, UNDIFFER-TYPE			3 (16%)		
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE			1 (5%)		
MALIG.LYMPHOMA, HISTIOCYTIC TYPE			1 (5%)		1 (10%)
LYMPHOCYTIC LEUKEMIA	3 (30%)			1 (10%)	
#DUODENUM	(10)	(10)	(19)	(10)	(10)
MALIG.LYMPHOMA, UNDIFFER-TYPE			1 (5%)		
CIRCULATORY SYSTEM					
NONE					
DIGESTIVE SYSTEM					
#LIVER	(10)	(10)	(19)	(10)	(10)
HEPATOCELLULAR ADENOMA			1 (5%)		
URINARY SYSTEM					
NONE					

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B3 CONTROL FEMALE MICE: NEOPLASMS (CONTINUED)

	LOWER MID DOSE UNTREATED CONTROL	UPPER MID AND HIGH DOSE UNTREATED CONTROL	LOW DOSE UNTREATED CONTROL	LOWER MID DOSE VEHICLE CONTROL	UPPER MID AND HIGH DOSE VEHICLE CONTROL
ENDOCRINE SYSTEM					
#PITUITARY	(7)	(9)	(17)	(8)	(9)
CHROMOPHOBE ADENOMA			3 (18%)		
REPRODUCTIVE SYSTEM					
#UTERUS	(10)	(9)	(19)	(10)	(9)
HEMANGIOSARCOMA		1 (11%)			
NERVOUS SYSTEM					
NONE					
SPECIAL SENSE ORGANS					
*HARDERIAN GLAND	(10)	(10)	(19)	(10)	(10)
PAPILLARY CYSTADENOMA, NOS			1 (5%)		
MUSCULOSKELETAL SYSTEM					
NONE					
BODY CAVITIES					
NONE					
ALL OTHER SYSTEMS					
*MULTIPLE ORGANS	(10)	(10)	(19)	(10)	(10)
FIBROSARCOMA			1 (5%)		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B3 CONTROL FEMALE MICE: NEOPLASMS (CONTINUED)

	LOWER MID DOSE UNTREATED CONTROL	UPPER MID AND HIGH DOSE UNTREATED CONTROL	LOW DOSE UNTREATED CONTROL	LOWER MID DOSE VEHICLE CONTROL	UPPER MID AND HIGH DOSE VEHICLE CONTROL
ANIMAL DISPOSITION SUMMARY					
ANIMALS INITIALLY IN STUDY	10	10	20	10	10
NATURAL DEATH@		1	6	1	
MORIBUND SACRIFICE			4	1	1
SCHEDULED SACRIFICE					
ACCIDENTALLY KILLED					
TERMINAL SACRIFICE	10	9	10	8	9
ANIMAL MISSING					

@ INCLUDES AUTOLYZED ANIMALS

	LOWER MID DOSE UNTREATED CONTROL	UPPER MID AND HIGH DOSE UNTREATED CONTROL	LOW DOSE UNTREATED CONTROL	LOWER MID DOSE VEHICLE CONTROL	UPPER MID AND HIGH DOSE VEHICLE CONTROL
TUMOR SUMMARY					
TOTAL ANIMALS WITH PRIMARY TUMORS*	3	1	13	1	1
TOTAL PRIMARY TUMORS	3	1	15	1	1
TOTAL ANIMALS WITH BENIGN TUMORS			7		
TOTAL BENIGN TUMORS			7		
TOTAL ANIMALS WITH MALIGNANT TUMORS	3	1	8	1	1
TOTAL MALIGNANT TUMORS	3	1	8	1	1
TOTAL ANIMALS WITH SECONDARY TUMORS#					
TOTAL SECONDARY TUMORS					
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT					
TOTAL UNCERTAIN TUMORS					
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC					
TOTAL UNCERTAIN TUMORS					

* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS

# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN

TABLE B4

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE GIVEN INTRAPERITONEAL INJECTIONS OF ACRONYCINE (CONTROL AND TREATED GROUPS)

	LOW DOSE VEHICLE CONTROL	LOW DOSE	LOWER MID DOSE	UPPER MID DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	40	35	35	35
ANIMALS NECROPSIED	20	39	33	33	32
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	39	33	33	15
INTEGUMENTARY SYSTEM					
NONE					
RESPIRATORY SYSTEM					
#LUNG	(17)	(38)	(33)	(33)	(15)
ALVEOLAR/BRONCHIOLAR CARCINOMA		1 (3%)			
HEMATOPOIETIC SYSTEM					
*MULTIPLE ORGANS	(20)	(39)	(33)	(33)	(32)
MALIGNANT LYMPHOMA, NOS	4 (20%)				
MALIG.LYMPHOMA, UNDIFFER-TYPE	13 (65%)	1 (3%)			
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE	2 (10%)	4 (10%)			
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		1 (3%)			
#BONE MARROW	(18)	(39)	(31)	(32)	(14)
GRANULOCYTIC LEUKEMIA				1 (3%)	
#THYMUS	(9)	(11)	(22)	(7)	(4)
ALVEOLAR/BRONCHIOLAR CA, METASTA		1 (9%)			
CIRCULATORY SYSTEM					
NONE					
DIGESTIVE SYSTEM					
#LIVER	(20)	(39)	(31)	(32)	(15)
HEPATOCELLULAR CARCINOMA		1 (3%)			
*BILE DUCT	(20)	(39)	(33)	(33)	(32)
ADENOCARCINOMA, NOS				1 (3%)	

# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B4 CONTROL & TREATED FEMALE MICE: NEOPLASMS (CONTINUED)

	LOW DOSE VEHICLE CONTROL	LOW DOSE	LOWER MID DOSE	UPPER MID DOSE	HIGH DOSE
#DUODENUM	(12)	(39)	(28)	(32)	(6)
ADENOMATOUS POLYP, NOS		1 (3%)			
URINARY SYSTEM					
NONE					
ENDOCRINE SYSTEM					
NONE					
REPRODUCTIVE SYSTEM					
*MAMMARY GLAND	(20)	(39)	(33)	(33)	(32)
ADENOCARCINOMA, NOS		1 (3%)			
NERVOUS SYSTEM					
NONE					
SPECIAL SENSE ORGANS					
NONE					
MUSCULOSKELETAL SYSTEM					
NONE					
BODY CAVITIES					
NONE					
ALL OTHER SYSTEMS					
*MULTIPLE ORGANS	(20)	(39)	(33)	(33)	(32)
SARCOMA, NOS		2 (5%)			

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B4 CONTROL & TREATED FEMALE MICE: NEOPLASMS (CONTINUED)

	LOW DOSE VEHICLE CONTROL	LOW DOSE	LOWER MID DOSE	UPPER MID DOSE	HIGH DOSE
ANIMAL DISPOSITION SUMMARY					
ANIMALS INITIALLY IN STUDY	20	40	35	35	35
NATURAL DEATH@	14	7	14	12	29
MORIBUND SACRIFICE	6	31	21	23	6
SCHEDULED SACRIFICE					
ACCIDENTALLY KILLED		2			
TERMINAL SACRIFICE					
ANIMAL MISSING					

@ INCLUDES AUTOLYZED ANIMALS

	LOW DOSE VEHICLE CONTROL	LOW DOSE	LOWER MID DOSE	UPPER MID DOSE	HIGH DOSE
TUMOR SUMMARY					
TOTAL ANIMALS WITH PRIMARY TUMORS*	19	9		2	
TOTAL PRIMARY TUMORS	19	12		2	
TOTAL ANIMALS WITH BENIGN TUMORS		1			
TOTAL BENIGN TUMORS		1			
TOTAL ANIMALS WITH MALIGNANT TUMORS	19	9		2	
TOTAL MALIGNANT TUMORS	19	11		2	
TOTAL ANIMALS WITH SECONDARY TUMORS#		1			
TOTAL SECONDARY TUMORS		1			
TOTAL ANIMALS WITH TUMORS UNCERTAIN- BENIGN OR MALIGNANT					
TOTAL UNCERTAIN TUMORS					
TOTAL ANIMALS WITH TUMORS UNCERTAIN- PRIMARY OR METASTATIC					
TOTAL UNCERTAIN TUMORS					

* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS
# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN

APPENDIX C

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN RATS GIVEN INTRAPERITONEAL INJECTIONS OF ACRONYCINE

TABLE C1

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS GIVEN INTRAPERITONEAL INJECTIONS OF ACRONYCINE (CONTROL GROUPS)

	LOW DOSE UNTREATED CONTROL	MID AND HIGH DOSE UNTREATED CONTROL	LOW DOSE VEHICLE CONTROL	MID AND HIGH DOSE VEHICLE CONTROL
ANIMALS INITIALLY IN STUDY	10	10	10	10
ANIMALS NECROPSIED	10	9	10	8
ANIMALS EXAMINED HISTOPATHOLOGICALLY	10	9	10	8
INTEGUMENTARY SYSTEM				
NONE				
RESPIRATORY SYSTEM				
#TRACHEA	(9)	(9)	(8)	(8)
INFLAMMATION, CHRONIC		2 (22%)		1 (13%)
INFLAMMATION, CHRONIC SUPPURATIV				1 (13%)
#LUNG/BRONCHIOLE	(10)	(9)	(10)	(8)
HYPERPLASIA, LYMPHOID		3 (33%)		1 (13%)
#LUNG	(10)	(9)	(10)	(8)
BRONCHOPNEUMONIA, NOS				1 (13%)
BRONCHOPNEUMONIA SUPPURATIVE		1 (11%)		2 (25%)
PNEUMONIA, CHRONIC MURINE	5 (50%)		7 (70%)	
HEMATOPOIETIC SYSTEM				
#BONE MARROW	(9)	(9)	(10)	(8)
ATROPHY, NOS	1 (11%)	4 (44%)	2 (20%)	2 (25%)
HYPERPLASIA, NOS				1 (13%)
#MANDIBULAR L. NODE	(7)	(9)	(6)	(7)
HYPERPLASIA, PLASMA CELL			1 (17%)	
CIRCULATORY SYSTEM				
#ENDOCARDIUM	(9)	(9)	(10)	(6)
FIBROSIS, FOCAL			1 (10%)	
*CELIAC ARTERY	(10)	(9)	(10)	(8)
DEGENERATION, NOS		1 (11%)		

# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C1 CONTROL MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	LOW DOSE UNTREATED CONTROL	MID AND HIGH DOSE UNTREATED CONTROL	LOW DOSE VEHICLE CONTROL	MID AND HIGH DOSE VEHICLE CONTROL
DIGESTIVE SYSTEM				
#SMALL INTESTINE	(8)	(9)	(10)	(6)
PERIARTERITIS		1 (11%)		
URINARY SYSTEM				
#KIDNEY	(9)	(9)	(10)	(8)
CALCULUS, NOS		2 (22%)		1 (13%)
INFLAMMATION, INTERSTITIAL				1 (13%)
INFLAMMATION, CHRONIC	3 (33%)	3 (33%)	7 (70%)	2 (25%)
#KIDNEY/TUBULE	(9)	(9)	(10)	(8)
MINERALIZATION		1 (11%)		
ENDOCRINE SYSTEM				
NONE				
REPRODUCTIVE SYSTEM				
#PROSTATE	(9)	(7)	(10)	(8)
INFLAMMATION, SUPPURATIVE		2 (29%)		
NERVOUS SYSTEM				
NONE				
SPECIAL SENSE ORGANS				
NONE				
MUSCULOSKELETAL SYSTEM				
NONE				
BODY CAVITIES				
NONE				

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C1 CONTROL MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	LOW DOSE UNTREATED CONTROL	MID AND HIGH DOSE UNTREATED CONTROL	LOW DOSE VEHICLE CONTROL	MID AND HIGH DOSE VEHICLE CONTROL
ALL OTHER SYSTEMS				
NONE				
SPECIAL MORPHOLOGY SUMMARY				
NO LESION REPORTED	3	1	1	1
AUTOLYSIS/NO NECROPSY		1		2

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C2

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS GIVEN INTRAPERITONEAL INJECTIONS OF ACRONYCINE (TREATED GROUPS)

	LOW DOSE	MID DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	35	35	35
ANIMALS NECROPSIED	31	32	34
ANIMALS EXAMINED HISTOPATHOLOGICALLY	30	31	34
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(31)	(32)	(34)
EPIDERMAL INCLUSION CYST	1 (3%)		
HEMORRHAGE			1 (3%)
INFLAMMATION, NECROTIZING	1 (3%)		
INFLAMMATION, FOCAL GRANULOMATOU	1 (3%)		
RESPIRATORY SYSTEM			
#TRACHEA	(29)	(31)	(31)
INFLAMMATION, SUPPURATIVE	2 (7%)	1 (3%)	2 (6%)
INFLAMMATION, ACUTE/CHRONIC		2 (6%)	4 (13%)
INFLAMMATION, CHRONIC SUPPURATIV	1 (3%)		
#LUNG/BRONCHUS	(30)	(31)	(34)
INFLAMMATION, NOS		1 (3%)	
#LUNG	(30)	(31)	(34)
HEMORRHAGE			2 (6%)
BRONCHOPNEUMONIA, NOS			1 (3%)
INFLAMMATION, INTERSTITIAL		1 (3%)	
BRONCHOPNEUMONIA SUPPURATIVE	1 (3%)	3 (10%)	1 (3%)
PNEUMONIA, CHRONIC MURINE	8 (27%)		
HYPERPLASIA, ALVEOLAR EPITHELIUM		1 (3%)	
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(28)	(29)	(32)
ATROPHY, NOS	6 (21%)	8 (28%)	19 (59%)
#SPLEEN	(27)	(31)	(33)
FIBROSIS		1 (3%)	
HEMATOPOIESIS	3 (11%)		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C2 TREATED MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	LOW DOSE	MID DOSE	HIGH DOSE
#MEDIASTINAL L.NODE	(30)	(20)	(21)
HEMORRHAGE			1 (5%)
#PANCREATIC L.NODE	(30)	(20)	(21)
HYPERPLASIA, LYMPHOID	1 (3%)		
#MESENTERIC L. NODE	(30)	(20)	(21)
CONGESTION, NOS		1 (5%)	
HEMORRHAGE		1 (5%)	
HYPERPLASIA, LYMPHOID	1 (3%)		
CIRCULATORY SYSTEM			
#MYOCARDIUM	(29)	(30)	(33)
HEMORRHAGE	1 (3%)		
INFLAMMATION, INTERSTITIAL	1 (3%)		
INFLAMMATION, CHRONIC FOCAL	1 (3%)		
INFLAMMATION, CHRONIC SUPPURATIV	1 (3%)		
*PULMONARY ARTERY	(31)	(32)	(34)
ARTERIOSCLEROSIS, NOS	1 (3%)		
DIGESTIVE SYSTEM			
#LIVER	(29)	(31)	(34)
HEMORRHAGE	1 (3%)	2 (6%)	1 (3%)
INFLAMMATION, NECROTIZING	1 (3%)		
INFLAMMATION, CHRONIC			1 (3%)
INFLAMMATION, CHRONIC NECROTIZIN	1 (3%)		
FIBROSIS		1 (3%)	
NECROSIS, NOS			1 (3%)
NECROSIS, COAGULATIVE	4 (14%)		1 (3%)
CYTOLOGIC DEGENERATION	1 (3%)		
HYPERPLASIA, NODULAR	5 (17%)		
HYPERPLASIA, LYMPHOID	1 (3%)		
#LIVER/PERIPORTAL	(29)	(31)	(34)
FIBROSIS, DIFFUSE			1 (3%)
*BILE DUCT	(31)	(32)	(34)
FIBROSIS, FOCAL	1 (3%)		
HYPERPLASIA, NOS	2 (6%)		
#PANCREAS	(24)	(26)	(30)
INFLAMMATION, INTERSTITIAL			1 (3%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C2 TREATED MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	LOW DOSE	MID DOSE	HIGH DOSE
FIBROSIS			1 (3%)
HYPERPLASIA, NOS			1 (3%)
METAPLASIA, OSSEOUS		1 (4%)	
#COLON	(24)	(29)	(28)
INFLAMMATION, HEMORRHAGIC			1 (4%)
#CECUM	(24)	(29)	(28)
HEMORRHAGE			1 (4%)
URINARY SYSTEM			
#KIDNEY	(29)	(31)	(33)
INFLAMMATION, CHRONIC	7 (24%)		
#URINARY BLADDER	(24)	(29)	(33)
FIBROSIS			1 (3%)
ENDOCRINE SYSTEM			
#ADRENAL CORTEX	(28)	(31)	(33)
HYPERPLASIA, NODULAR			2 (6%)
#THYROID	(26)	(26)	(22)
CYSTIC FOLLICLES	1 (4%)		
REPRODUCTIVE SYSTEM			
#PROSTATE	(26)	(31)	(32)
INFLAMMATION, SUPPURATIVE		1 (3%)	
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
*BONE	(31)	(32)	(34)
OSTEOPETROSIS			1 (3%)
HYPERPLASIA, NOS			1 (3%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C2 TREATED MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	LOW DOSE	MID DOSE	HIGH DOSE
BODY CAVITIES			
*PERITONEUM	(31)	(32)	(34)
INFLAMMATION, SUPPURATIVE			1 (3%)
ABSCESS, NOS			1 (3%)
INFLAMMATION, CHRONIC	1 (3%)		5 (15%)
INFLAMMATION, CHRONIC DIFFUSE	1 (3%)		
FIBROSIS		1 (3%)	2 (6%)
ADHESION, NOS	1 (3%)		
METAPLASIA, OSSEOUS	1 (3%)	1 (3%)	
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(31)	(32)	(34)
FIBROSIS		1 (3%)	
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED		4	1
NO NECROPSY PERFORMED	1		
AUTO/NECROPSY/NO HISTO	1	1	
AUTOLYSIS/NO NECROPSY	3	3	1

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C3

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS GIVEN INTRAPERITONEAL INJECTIONS OF ACRONYCINE (CONTROL GROUPS)

	LOW DOSE UNTREATED CONTROL	MID AND HIGH DOSE UNTREATED CONTROL	LOW DOSE VEHICLE CONTROL	MID AND HIGH DOSE VEHICLE CONTROL
ANIMALS INITIALLY IN STUDY	10	10	10	10
ANIMALS NECROPSIED	10	9	10	9
ANIMALS EXAMINED HISTOPATHOLOGICALLY	10	8	10	9
INTEGUMENTARY SYSTEM				
*SUBCUT TISSUE	(10)	(9)	(10)	(9)
INFLAMMATION, CHRONIC FOCAL			1 (10%)	
RESPIRATORY SYSTEM				
#TRACHEA	(10)	(8)	(10)	(9)
INFLAMMATION, NOS		1 (13%)		
INFLAMMATION, ACUTE/CHRONIC		2 (25%)		
#LUNG/BRONCHIOLE	(10)	(7)	(10)	(9)
HYPERPLASIA, LYMPHOID				2 (22%)
#LUNG	(10)	(7)	(10)	(9)
INFLAMMATION, INTERSTITIAL		1 (14%)		
PNEUMONIA, CHRONIC MURINE	1 (10%)		1 (10%)	
HEMATOPOIETIC SYSTEM				
#BONE MARROW	(10)	(8)	(9)	(9)
ATROPHY, NOS		5 (63%)		4 (44%)
#SPLEEN	(10)	(8)	(10)	(9)
HEMATOPOIESIS	1 (10%)			
CIRCULATORY SYSTEM				
NONE				
DIGESTIVE SYSTEM				
#HEPATIC CAPSULE	(10)	(8)	(9)	(9)
NECROSIS, COAGULATIVE			1 (11%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY

* NUMBER OF ANIMALS NECROPSIED

TABLE C3 CONTROL FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	LOW DOSE UNTREATED CONTROL	MID AND HIGH DOSE UNTREATED CONTROL	LOW DOSE VEHICLE CONTROL	MID AND HIGH DOSE VEHICLE CONTROL
URINARY SYSTEM				
#KIDNEY	(10)	(8)	(10)	(9)
CALCULUS, NOS		3 (38%)		
INFLAMMATION, CHRONIC	2 (20%)		4 (40%)	1 (11%)
ENDOCRINE SYSTEM				
NONE				
REPRODUCTIVE SYSTEM				
*MAMMARY GLAND	(10)	(9)	(10)	(9)
CYST, NOS		1 (11%)		
#UTERUS/ENDOMETRIUM	(10)	(8)	(10)	(9)
INFLAMMATION, SUPPURATIVE	3 (30%)	3 (38%)	2 (20%)	2 (22%)
INFLAMMATION, CHRONIC SUPPURATIV	1 (10%)			
NERVOUS SYSTEM				
NONE				
SPECIAL SENSE ORGANS				
NONE				
MUSCULOSKELETAL SYSTEM				
NONE				
BODY CAVITIES				
*PERITONEUM	(10)	(9)	(10)	(9)
INFLAMMATION, CHRONIC SUPPURATIV	1 (10%)			
ALL OTHER SYSTEMS				
NONE				

# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C3 CONTROL FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	LOW DOSE UNTREATED CONTROL	MID AND HIGH DOSE UNTREATED CONTROL	LOW DOSE VEHICLE CONTROL	MID AND HIGH DOSE VEHICLE CONTROL
SPECIAL MORPHOLOGY SUMMARY				
NO LESION REPORTED	2		3	2
NECROPSY PERF/NO HISTO PERFORMED		1		
AUTOLYSIS/NO NECROPSY		1		1

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

NIH LIBRARY

TABLE C4

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS GIVEN INTRAPERITONEAL INJECTIONS OF ACRONYCINE (TREATED GROUPS)

	LOW DOSE	MID DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	35	35	35
ANIMALS NECROPSIED	35	32	34
ANIMALS EXAMINED HISTOPATHOLOGICALLY	35	32	33
INTEGUMENTARY SYSTEM			
*SKIN	(35)	(32)	(34)
ULCER, CHRONIC	1 (3%)		
RESPIRATORY SYSTEM			
#TRACHEA	(35)	(30)	(32)
INFLAMMATION, NOS		1 (3%)	
INFLAMMATION, ACUTE/CHRONIC			1 (3%)
INFLAMMATION, CHRONIC		2 (7%)	1 (3%)
#LUNG/BRONCHUS	(35)	(32)	(33)
BRONCHIECTASIS		1 (3%)	
INFLAMMATION, NOS		1 (3%)	
#LUNG	(35)	(32)	(33)
HEMORRHAGE			1 (3%)
INFLAMMATION, INTERSTITIAL		2 (6%)	
BRONCHOPNEUMONIA SUPPURATIVE		2 (6%)	1 (3%)
PNEUMONIA, CHRONIC MURINE	5 (14%)		
INFLAMMATION, CHRONIC		1 (3%)	
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(35)	(32)	(31)
ATROPHY, NOS	3 (9%)	7 (22%)	10 (32%)
#SPLEEN	(35)	(31)	(31)
HEMATOPOIESIS	10 (29%)	1 (3%)	
#AXILLARY LYMPH NODE	(35)	(12)	(26)
HYPERPLASIA, PLASMA CELL	1 (3%)		
CIRCULATORY SYSTEM			
NONE			

# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C4 TREATED FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	LOW DOSE	MID DOSE	HIGH DOSE
DIGESTIVE SYSTEM			
#LIVER	(35)	(32)	(33)
HEMORRHAGIC CYST		1 (3%)	
INFLAMMATION, CHRONIC		1 (3%)	
FIBROSIS, DIFFUSE			1 (3%)
NECROSIS, NOS		1 (3%)	
NECROSIS, COAGULATIVE	1 (3%)		3 (9%)
HYPERPLASIA, NODULAR	1 (3%)	1 (3%)	
#LIVER/CENTRILOBULAR	(35)	(32)	(33)
NECROSIS, COAGULATIVE	1 (3%)		
*BILE DUCT	(35)	(32)	(34)
CYST, NOS	1 (3%)		
HYPERPLASIA, NOS		1 (3%)	1 (3%)
HYPERPLASIA, CYSTIC		1 (3%)	1 (3%)
#PANCREAS	(34)	(30)	(31)
FIBROSIS		1 (3%)	
#STOMACH	(35)	(30)	(32)
FIBROSIS		1 (3%)	
URINARY SYSTEM			
#KIDNEY	(35)	(31)	(32)
CALCULUS, NOS		1 (3%)	
HYDRONEPHROSIS	1 (3%)		
INFLAMMATION, SUPPURATIVE			1 (3%)
INFLAMMATION, CHRONIC	3 (9%)	1 (3%)	
#KIDNEY/TUBULE	(35)	(31)	(32)
NEPHROSIS, NOS			1 (3%)
ENDOCRINE SYSTEM			
#ADRENAL	(32)	(30)	(31)
INFLAMMATION, CHRONIC		1 (3%)	
ANGIECTASIS	1 (3%)	2 (7%)	
#ADRENAL CORTEX	(32)	(30)	(31)
HYPERPLASIA, NODULAR			1 (3%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C4 TREATED FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	LOW DOSE	MID DOSE	HIGH DOSE
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(35)	(32)	(34)
INFLAMMATION, NECROTIZING	1 (3%)		
HYPERPLASIA, CYSTIC		1 (3%)	1 (3%)
*VAGINA	(35)	(32)	(34)
INFLAMMATION, SUPPURATIVE			1 (3%)
#UTERUS/ENDOMETRIUM	(34)	(32)	(32)
INFLAMMATION, SUPPURATIVE	6 (18%)	1 (3%)	
HYPERPLASIA, CYSTIC	2 (6%)		
#OVARY/OVIDUCT	(34)	(32)	(32)
HEMORRHAGE	1 (3%)		
#OVARY	(31)	(31)	(31)
INFLAMMATION, SUPPURATIVE	1 (3%)		
INFLAMMATION, CHRONIC		1 (3%)	
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*PERITONEUM	(35)	(32)	(34)
INFLAMMATION, CHRONIC		2 (6%)	2 (6%)
FIBROSIS		1 (3%)	1 (3%)
ALL OTHER SYSTEMS			
DIAPHRAGM			
INFLAMMATION, CHRONIC		1	

# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C4 TREATED FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	LOW DOSE	MID DOSE	HIGH DOSE
ADIPOSE TISSUE			
INFLAMMATION, CHRONIC FOCAL	1		
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	1	1	1
AUTO/NECROPSY/NO HISTO			1
AUTOLYSIS/NO NECROPSY		3	1

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

NIH LIBRARY

APPENDIX D

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MICE GIVEN INTRAPERITONEAL INJECTIONS OF ACRONYCINE

TABLE D1

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE GIVEN INTRAPERITONEAL INJECTIONS OF ACRONYCINE (CONTROL GROUPS)

	LOWER MID DOSE UNTREATED CONTROL	UPPER MID AND HIGH DOSE UNTREATED CONTROL	LOW DOSE UNTREATED CONTROL	LOWER MID DOSE VEHICLE CONTROL	UPPER MID AND HIGH DOSE VEHICLE CONTROL
ANIMALS INITIALLY IN STUDY	10	10	20	10	10
ANIMALS NECROPSIED	9	10	20	10	10
ANIMALS EXAMINED HISTOPATHOLOGICALLY	9	10	20	10	10
INTEGUMENTARY SYSTEM					
*SKIN	(9)	(10)	(20)	(10)	(10)
ULCER, FOCAL			1 (5%)		
FIBROSIS			1 (5%)		
FIBROSIS, FOCAL			2 (10%)		
ACARIASIS			1 (5%)		
*SUBCUT TISSUE	(9)	(10)	(20)	(10)	(10)
GRANULATION, TISSUE			1 (5%)		
RESPIRATORY SYSTEM					
#LUNG	(9)	(10)	(20)	(10)	(10)
INFLAMMATION, INTERSTITIAL				1 (10%)	
BRONCHOPNEUMONIA SUPPURATIVE	1 (11%)				
HYPERPLASIA, ALVEOLAR EPITHELIUM			1 (5%)		
HYPERPLASIA, LYMPHOID			2 (10%)		
HEMATOPOIETIC SYSTEM					
#SPLEEN	(9)	(10)	(20)	(9)	(10)
ATROPHY, NOS					1 (10%)
HYPERPLASIA, HEMATOPOIETIC	1 (11%)			1 (11%)	
HYPERPLASIA, LYMPHOID				1 (11%)	
HEMATOPOIESIS			3 (15%)		
#LYMPH NODE	(9)	(9)	(20)	(10)	(9)
ATROPHY, NOS					1 (11%)
#MEDIASTINAL L.NODE	(9)	(9)	(20)	(10)	(9)
ATROPHY, NOS				1 (10%)	
#PANCREATIC L.NODE	(9)	(9)	(20)	(10)	(9)
HYPERPLASIA, LYMPHOID				1 (10%)	

CONTINUED ON

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D1 CONTROL MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	LOWER MID DOSE UNTREATED CONTROL	UPPER MID AND HIGH DOSE UNTREATED CONTROL	LOW DOSE UNTREATED CONTROL	LOWER MID DOSE VEHICLE CONTROL	UPPER MID AND HIGH DOSE VEHICLE CONTROL
#MESENTERIC L. NODE	(9)	(9)	(20)	(10)	(9)
HEMORRHAGE	1 (11%)				
ATROPHY, NOS	1 (11%)				
ANGIECTASIS	1 (11%)				
#THYMUS	(3)		(4)	(1)	(1)
ATROPHY, NOS				1 (100%)	1 (100%)
CIRCULATORY SYSTEM					
#MYOCARDIUM	(9)	(10)	(20)	(10)	(10)
INFLAMMATION, INTERSTITIAL			1 (5%)	1 (10%)	
DIGESTIVE SYSTEM					
#LIVER	(9)	(10)	(20)	(10)	(10)
NECROSIS, NOS				1 (10%)	
HYPERTROPHY, NOS		1 (10%)			
HYPERPLASIA, NODULAR			1 (5%)		
HYPERPLASTIC NODULE	2 (22%)				
HYPERPLASIA, HEMATOPOIETIC				2 (20%)	
HYPERPLASIA, LYMPHOID				2 (20%)	
#LIVER/CENTRILOBULAR	(9)	(10)	(20)	(10)	(10)
DEGENERATION, NOS				1 (10%)	
#LIVER/HEPATOCYTES	(9)	(10)	(20)	(10)	(10)
HYPERPLASIA, NOS		1 (10%)			
URINARY SYSTEM					
#KIDNEY	(9)	(10)	(20)	(10)	(10)
HYDRONEPHROSIS					1 (10%)
INFLAMMATION, CHRONIC			2 (10%)		
INFARCT, NOS			1 (5%)		
#URINARY BLADDER	(9)	(10)	(20)	(8)	(10)
INFLAMMATION, CHRONIC					2 (20%)
ENDOCRINE SYSTEM					
#ADRENAL	(8)	(10)	(18)	(10)	(9)
FIBROSIS				1 (10%)	
CALCIFICATION, NOS				1 (10%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY

NIH LIBRARY

TABLE D1 CONTROL MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	LOWER MID DOSE UNTREATED CONTROL	UPPER MID AND HIGH DOSE UNTREATED CONTROL	LOW DOSE UNTREATED CONTROL	LOWER MID DOSE VEHICLE CONTROL	UPPER MID AND HIGH DOSE VEHICLE CONTROL
REPRODUCTIVE SYSTEM					
#PROSTATE	(2)	(10)	(20)	(10)	(10)
INFLAMMATION, SUPPURATIVE				1 (10%)	
INFLAMMATION, CHRONIC SUPPURATIV					1 (10%)
#TESTIS	(9)	(10)	(20)	(10)	(10)
CALCIFICATION, NOS				1 (10%)	
NERVOUS SYSTEM					
NONE					
SPECIAL SENSE ORGANS					
NONE					
MUSCULOSKELETAL SYSTEM					
NONE					
BODY CAVITIES					
NONE					
ALL OTHER SYSTEMS					
NONE					
SPECIAL MORPHOLOGY SUMMARY					
NO LESION REPORTED	5	5	4	2	7
AUTOLYSIS/NO NECROPSY	1				

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D2

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE GIVEN INTRAPERITONEAL INJECTIONS OF ACRONYCINE (CONTROL AND TREATED GROUPS)

	LOW DOSE VEHICLE CONTROL	LOW DOSE	LOWER MID DOSE	UPPER MID DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	40	35	35	35
ANIMALS MISSING				1	
ANIMALS NECROPSIED	20	40	35	34	29
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	40	35	33	12
INTEGUMENTARY SYSTEM					
*SKIN	(20)	(40)	(35)	(34)	(29)
HEMATOMA, NOS					1 (3%)
*SUBCUT TISSUE	(20)	(40)	(35)	(34)	(29)
HEMORRHAGE					1 (3%)
RESPIRATORY SYSTEM					
#TRACHEA	(16)	(38)	(31)	(29)	(11)
INFLAMMATION, SUPPURATIVE	1 (6%)				
#LUNG	(18)	(40)	(35)	(33)	(12)
HEMORRHAGE			2 (6%)		1 (8%)
INFLAMMATION, INTERSTITIAL	2 (11%)	1 (3%)			
BRONCHOPNEUMONIA SUPPURATIVE	1 (6%)	2 (5%)	1 (3%)		
HYPERPLASIA, LYMPHOID		1 (3%)			
HEMATOPOIETIC SYSTEM					
#BONE MARROW	(20)	(38)	(34)	(33)	(12)
ATROPHY, NOS			1 (3%)		1 (8%)
HYPERPLASIA, HEMATOPOIETIC			1 (3%)		
HYPERPLASIA, GRANULOCYTIC		1 (3%)			
#SPLEEN	(19)	(38)	(35)	(30)	(12)
ATROPHY, NOS			2 (6%)	1 (3%)	1 (8%)
#MEDIASTINAL L.NODE	(17)	(40)	(31)	(31)	(10)
HEMORRHAGE					1 (10%)
ATROPHY, NOS				2 (6%)	7 (70%)
#MESENTERIC L. NODE	(17)	(40)	(31)	(31)	(10)
ATROPHY, NOS			1 (3%)		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D2 CONTROL AND TREATED MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	LOW DOSE VEHICLE CONTROL	LOW DOSE	LOWER MID DOSE	UPPER MID DOSE	HIGH DOSE
HYPERPLASIA, LYMPHOID		1 (3%)			
#THYMUS	(5)	(7)	(15)	(2)	(9)
ATROPHY, NOS			15 (100%)	2 (100%)	9 (100%)
CIRCULATORY SYSTEM					
#MYOCARDIUM	(20)	(39)	(35)	(32)	(12)
INFLAMMATION, SUPPURATIVE		3 (8%)	2 (6%)		
INFLAMMATION, CHRONIC DIFFUSE		1 (3%)			
#ENDOCARDIUM	(20)	(39)	(35)	(32)	(12)
INFLAMMATION, NOS		2 (5%)			
INFLAMMATION, FOCAL		1 (3%)			
INFLAMMATION, SUPPURATIVE			3 (9%)		
INFLAMMATION, ACUTE/CHRONIC			1 (3%)		
*AORTA	(20)	(40)	(35)	(34)	(29)
INFLAMMATION, SUPPURATIVE			1 (3%)		
DIGESTIVE SYSTEM					
#LIVER	(20)	(39)	(34)	(33)	(12)
THROMBOSIS, NOS			1 (3%)		
INFLAMMATION, SUPPURATIVE			1 (3%)		
ABSCESS, NOS			1 (3%)		
INFLAMMATION, CHRONIC SUPPURATIV			1 (3%)		
NECROSIS, FOCAL			1 (3%)		
NECROSIS, COAGULATIVE		3 (8%)	1 (3%)		
HYPERPLASIA, NODULAR	1 (5%)				
ANGIECTASIS		1 (3%)			
HYPERPLASIA, HEMATOPOIETIC			1 (3%)		
HYPERPLASIA, LYMPHOID		1 (3%)			
HEMATOPOIESIS	1 (5%)				
*BILE DUCT	(20)	(40)	(35)	(34)	(29)
INFLAMMATION, SUPPURATIVE		1 (3%)	3 (9%)		
INFLAMMATION, CHRONIC SUPPURATIV			1 (3%)		
HYPERPLASIA, NOS		1 (3%)	5 (14%)	1 (3%)	
HYPERPLASIA, FOCAL			7 (20%)		1 (3%)
#PANCREAS	(20)	(38)	(33)	(30)	(12)
INFLAMMATION, INTERSTITIAL			2 (6%)		1 (8%)
ATROPHY, NOS			2 (6%)		

# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D2 CONTROL AND TREATED MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	LOW DOSE VEHICLE CONTROL	LOW DOSE	LOWER MID DOSE	UPPER MID DOSE	HIGH DOSE
ATROPHY, FOCAL					1 (8%)
HYPERPLASIA, NODULAR				1 (3%)	
#GASTRIC SUBMUCOSA	(20)	(38)	(32)	(30)	(12)
HEMORRHAGE					1 (8%)
URINARY SYSTEM					
#KIDNEY	(20)	(40)	(35)	(33)	(12)
PYELONEPHRITIS, FOCAL		2 (5%)			
INFLAMMATION, INTERSTITIAL			2 (6%)		1 (8%)
INFLAMMATION, SUPPURATIVE			2 (6%)		
INFLAMMATION, CHRONIC			1 (3%)		2 (17%)
INFLAMMATION, CHRONIC FOCAL			1 (3%)		
INFLAMMATION, CHRONIC SUPPURATIV			2 (6%)		
FIBROSIS, DIFFUSE					1 (8%)
NECROSIS, MEDULLARY		1 (3%)	6 (17%)		
#KIDNEY/CORTEX	(20)	(40)	(35)	(33)	(12)
ATROPHY, NOS					1 (8%)
ATROPHY, FOCAL					1 (8%)
#KIDNEY/TUBULE	(20)	(40)	(35)	(33)	(12)
CAST, NOS			1 (3%)		
#KIDNEY/PELVIS	(20)	(40)	(35)	(33)	(12)
ABSCESS, NOS					1 (8%)
#URINARY BLADDER	(20)	(38)	(33)	(33)	(12)
INFLAMMATION, SUPPURATIVE			1 (3%)		
HEMOSIDEROSIS					1 (8%)
ENDOCRINE SYSTEM					
NONE					
REPRODUCTIVE SYSTEM					
*SEMINAL VESICLE	(20)	(40)	(35)	(34)	(29)
INFLAMMATION, SUPPURATIVE			1 (3%)		
#TESTIS	(20)	(38)	(34)	(31)	(12)
FIBROSIS, DIFFUSE			1 (3%)		
ATROPHY, NOS					1 (8%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

NIH LIBRARY

TABLE D2 CONTROL AND TREATED MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	LOW DOSE VEHICLE CONTROL	LOW DOSE	LOWER MID DOSE	UPPER MID DOSE	HIGH DOSE
NERVOUS SYSTEM					
*BRAIN/MENINGES	(20)	(40)	(33)	(28)	(12)
INFLAMMATION, SUPPURATIVE			1 (3%)		
SPECIAL SENSE ORGANS					
NONE					
MUSCULOSKELETAL SYSTEM					
NONE					
BODY CAVITIES					
*PERITONEUM	(20)	(40)	(35)	(34)	(29)
INFLAMMATION, NOS			1 (3%)		
INFLAMMATION, SUPPURATIVE		1 (3%)	2 (6%)		
INFLAMMATION, FIBRINOUS			1 (3%)		
INFLAMMATION ACUTE AND CHRONIC		1 (3%)			
INFLAMMATION, ACUTE/CHRONIC		1 (3%)	1 (3%)		
INFLAMMATION, CHRONIC			21 (60%)		
INFLAMMATION, CHRONIC FOCAL			2 (6%)		
INFLAMMATION, CHRONIC DIFFUSE		1 (3%)			
INFLAMMATION, CHRONIC SUPPURATIV			1 (3%)		
INFLAMMATION, PYOGRANULOMATOUS					1 (3%)
FIBROSIS				2 (6%)	
FIBROSIS, FOCAL					1 (3%)
*PLEURA	(20)	(40)	(35)	(34)	(29)
INFLAMMATION, SUPPURATIVE			3 (9%)		
ALL OTHER SYSTEMS					
*MULTIPLE ORGANS	(20)	(40)	(35)	(34)	(29)
ATROPHY, NOS				1 (3%)	
HYPERPLASIA, LYMPHOID		1 (3%)			
SPECIAL MORPHOLOGY SUMMARY					
NO LESION REPORTED	4	13	3	29	1

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D2 CONTROL AND TREATED MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	LOW DOSE VEHICLE CONTROL	LOW DOSE	LOWER MID DOSE	UPPER MID DOSE	HIGH DOSE
ANIMAL MISSING/NO NECROPSY				1	
NECROPSY PERF/NO HISTO PERFORMED					17
AUTO/NECROPSY/NO HISTO				1	
AUTOLYSIS/NO NECROPSY					6

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D3

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE GIVEN INTRAPERITONEAL INJECTIONS OF ACRONYCINE (CONTROL GROUPS)

	LOWER MID DOSE UNTREATED CONTROL	UPPER MID AND HIGH DOSE UNTREATED CONTROL	LOW DOSE UNTREATED CONTROL	LOWER MID DOSE VEHICLE CONTROL	UPPER MID AND HIGH DOSE VEHICLE CONTROL
ANIMALS INITIALLY IN STUDY	10	10	20	10	10
ANIMALS NECROPSIED	10	10	19	10	10
ANIMALS EXAMINED HISTOPATHOLOGICALLY	10	10	19	10	10
INTEGUMENTARY SYSTEM					
*SKIN	(10)	(10)	(19)	(10)	(10)
ULCER, FOCAL			1 (5%)		
RESPIRATORY SYSTEM					
#LUNG/BRONCHUS	(10)	(10)	(19)	(10)	(9)
BRONCHIECTASIS				1 (10%)	
INFLAMMATION, SUPPURATIVE				1 (10%)	
HYPERPLASIA, LYMPHOID	1 (10%)				
#LUNG/BRONCHIOLE	(10)	(10)	(19)	(10)	(9)
HYPERPLASIA, LYMPHOID				1 (10%)	
#LUNG	(10)	(10)	(19)	(10)	(9)
EDEMA, NOS				1 (10%)	
BRONCHOPNEUMONIA, NOS				1 (10%)	
INFLAMMATION, INTERSTITIAL	6 (60%)		1 (5%)	4 (40%)	
HYPERPLASIA, ALVEOLAR EPITHELIUM	1 (10%)				
HYPERPLASIA, LYMPHOID	2 (20%)		6 (32%)	1 (10%)	
HEMATOPOIETIC SYSTEM					
#SPLEEN	(10)	(10)	(19)	(10)	(10)
NECROSIS, COAGULATIVE	1 (10%)				
HYPERPLASIA, HEMATOPOIETIC				3 (30%)	
HYPERPLASIA, LYMPHOID			3 (16%)		
HEMATOPOIESIS			5 (26%)		
#MESENTERIC L. NODE	(10)	(1)	(19)	(10)	(10)
HYPERPLASIA, LYMPHOID				1 (10%)	
#THYMUS		(1)	(6)	(1)	
ATROPHY, NOS				1 (100%)	
HYPERPLASIA, LYMPHOID			1 (17%)		

# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D3 CONTROL FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	LOWER MID DOSE UNTREATED CONTROL	UPPER MID AND HIGH DOSE UNTREATED CONTROL	LOW DOSE UNTREATED CONTROL	LOWER MID DOSE VEHICLE CONTROL	UPPER MID AND HIGH DOSE VEHICLE CONTROL
CIRCULATORY SYSTEM					
#HEART	(10)	(10)	(19)	(10)	(9)
PERIARTERITIS		2 (20%)			
DIGESTIVE SYSTEM					
#LIVER	(10)	(10)	(19)	(10)	(10)
NECROSIS, COAGULATIVE	1 (10%)				
CYTOPLASMIC VACUOLIZATION			1 (5%)		
BASOPHILIC CYTO CHANGE	1 (10%)				
HYPERPLASTIC NODULE				1 (10%)	
HYPERPLASIA, HEMATOPOIETIC	1 (10%)			2 (20%)	
HYPERPLASIA, LYMPHOID	2 (20%)		1 (5%)		
*BILE DUCT	(10)	(10)	(19)	(10)	(10)
HYPERPLASIA, HEMATOPOIETIC				1 (10%)	
#PANCREAS	(10)	(9)	(19)	(10)	(10)
CYSTIC DUCTS			1 (5%)		
INFLAMMATION, INTERSTITIAL				1 (10%)	
#PANCREATIC ACINUS	(10)	(9)	(19)	(10)	(10)
ATROPHY, NOS			1 (5%)		
ATROPHY, FOCAL			1 (5%)		
URINARY SYSTEM					
#KIDNEY	(10)	(10)	(19)	(10)	(10)
INFLAMMATION, CHRONIC			1 (5%)		
INFARCT, NOS		1 (10%)			
#URINARY BLADDER	(10)	(9)	(19)	(8)	(9)
HYPERPLASIA, LYMPHOID				1 (13%)	
ENDOCRINE SYSTEM					
#THYROID	(5)	(8)	(19)	(6)	(10)
HYPERPLASIA, CYSTIC				1 (17%)	
HYPERPLASIA, FOLLICULAR-CELL			1 (5%)		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

NIH LIBRARY

TABLE D3 CONTROL FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	LOWER MID DOSE UNTREATED CONTROL	UPPER MID AND HIGH DOSE UNTREATED CONTROL	LOW DOSE UNTREATED CONTROL	LOWER MID DOSE VEHICLE CONTROL	UPPER MID AND HIGH DOSE VEHICLE CONTROL
REPRODUCTIVE SYSTEM					
#UTERUS	(10)	(9)	(19)	(10)	(9)
CYST, NOS	1 (10%)				
HEMORRHAGE		1 (11%)			
PYOMETRA				1 (10%)	
ANGIECTASIS		1 (11%)			
#UTERUS/ENDOMETRIUM	(10)	(9)	(19)	(10)	(9)
HYPERPLASIA, CYSTIC	8 (80%)		15 (79%)	9 (90%)	
#OVARY	(8)	(3)	(18)	(6)	(6)
MINERALIZATION			1 (6%)		
FOLLICULAR CYST, NOS			1 (6%)		
ATROPHY, NOS				2 (33%)	
NERVOUS SYSTEM					
NONE					
SPECIAL SENSE ORGANS					
NONE					
MUSCULOSKELETAL SYSTEM					
NONE					
BODY CAVITIES					
NONE					
ALL OTHER SYSTEMS					
ADIPOSE TISSUE					
INFLAMMATION, FOCAL			1		
SPECIAL MORPHOLOGY SUMMARY					
NO LESION REPORTED		7			9
AUTOLYSIS/NO NECROPSY			1		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D4

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE GIVEN INTRAPERITONEAL INJECTIONS OF ACRONYCINE (CONTROL AND TREATED GROUPS)

	LOW DOSE VEHICLE CONTROL	LOW DOSE	LOWER MID DOSE	UPPER MID DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	40	35	35	35
ANIMALS NECROPSIED	20	39	33	33	32
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	39	33	33	15
INTEGUMENTARY SYSTEM					
*SKIN	(20)	(39)	(33)	(33)	(32)
INFLAMMATION, SUPPURATIVE			1 (3%)		
*SUBCUT TISSUE	(20)	(39)	(33)	(33)	(32)
INFLAMMATION, SUPPURATIVE					1 (3%)
ABSCESS, NOS				1 (3%)	1 (3%)
RESPIRATORY SYSTEM					
#TRACHEA	(18)	(38)	(30)	(32)	(14)
INFLAMMATION, SUPPURATIVE		3 (8%)			
#LUNG	(17)	(38)	(33)	(33)	(15)
EDEMA, NOS			1 (3%)		
HEMORRHAGE			2 (6%)		2 (13%)
INFLAMMATION, INTERSTITIAL				1 (3%)	
BRONCHOPNEUMONIA SUPPURATIVE		11 (29%)	1 (3%)		
INFLAMMATION, ACUTE SUPPURATIVE			1 (3%)		
HYPERPLASIA, LYMPHOID		2 (5%)			
HEMATOPOIETIC SYSTEM					
#BONE MARROW	(18)	(39)	(31)	(32)	(14)
ATROPHY, NOS			1 (3%)	4 (13%)	
HYPERPLASIA, NOS				1 (3%)	
HYPERPLASIA, HEMATOPOIETIC				3 (9%)	
#SPLEEN	(20)	(39)	(33)	(31)	(15)
HEMORRHAGE				1 (3%)	
ATROPHY, NOS			5 (15%)	3 (10%)	1 (7%)
HYPERPLASIA, NOS				2 (6%)	
HYPERPLASIA, HEMATOPOIETIC				4 (13%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

NIH LIBRARY

TABLE D4. CONTROL AND TREATED FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	LOW DOSE VEHICLE CONTROL	LOW DOSE	LOWER MID DOSE	UPPER MID DOSE	HIGH DOSE
HYPERPLASIA, LYMPHOID		10 (26%)			
HEMATOPOIESIS		1 (3%)			
#MEDIASTINAL L.NODE	(20)	(37)	(28)	(31)	(12)
ATROPHY, NOS				5 (16%)	3 (25%)
HYPERPLASIA, PLASMA CELL				3 (10%)	
#THYMUS	(9)	(11)	(22)	(7)	(4)
ATROPHY, NOS			22 (100%)	6 (86%)	4 (100%)
HYPERPLASIA, PLASMA CELL				1 (14%)	
CIRCULATORY SYSTEM					
#MYOCARDIUM	(19)	(38)	(33)	(33)	(15)
INFLAMMATION, INTERSTITIAL		3 (8%)	1 (3%)		
INFLAMMATION, SUPPURATIVE			1 (3%)		
INFLAMMATION, ACUTE FOCAL				1 (3%)	
INFLAMMATION, ACUTE SUPPURATIVE			1 (3%)		
#ENDOCARDIUM	(19)	(38)	(33)	(33)	(15)
INFLAMMATION, NOS	1 (5%)	2 (5%)	1 (3%)		
INFLAMMATION, SUPPURATIVE			10 (30%)		
INFLAMMATION, FIBRINOUS			1 (3%)		
INFLAMMATION, ACUTE			2 (6%)		
*AORTA	(20)	(39)	(33)	(33)	(32)
INFLAMMATION, SUPPURATIVE			3 (9%)		
INFLAMMATION, ACUTE SUPPURATIVE			1 (3%)		
DIGESTIVE SYSTEM					
#LIVER	(20)	(39)	(31)	(32)	(15)
INFLAMMATION, SUPPURATIVE			1 (3%)	1 (3%)	
INFLAMMATION, NECROTIZING			2 (6%)		
INFLAMMATION, ACUTE SUPPURATIVE			1 (3%)		
NECROSIS, FOCAL				2 (6%)	
NECROSIS, COAGULATIVE	1 (5%)		4 (13%)		
LEUKOCYTOSIS, NOS				2 (6%)	
HYPERPLASIA, LYMPHOID		1 (3%)			
#LIVER/PERIPORTAL	(20)	(39)	(31)	(32)	(15)
NECROSIS, NOS				1 (3%)	
*BILE DUCT	(20)	(39)	(33)	(33)	(32)
DILATATION, NOS				1 (3%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D4. CONTROL AND TREATED FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	LOW DOSE VEHICLE CONTROL	LOW DOSE	LOWER MID DOSE	UPPER MID DOSE	HIGH DOSE
INFLAMMATION, SUPPURATIVE			2 (6%)	1 (3%)	
INFLAMMATION, ACUTE SUPPURATIVE				1 (3%)	
HYPERPLASIA, NOS			3 (9%)	19 (58%)	
HYPERPLASIA, FOCAL			12 (36%)		1 (3%)
HYPERPLASIA, DIFFUSE				1 (3%)	
HYPERPLASIA, CYSTIC			2 (6%)		
#PANCREAS	(16)	(35)	(30)	(29)	(15)
INFLAMMATION, FOCAL					1 (7%)
INFLAMMATION, INTERSTITIAL	1 (6%)		1 (3%)		
NECROSIS, COAGULATIVE			1 (3%)		
ATROPHY, NOS			1 (3%)	1 (3%)	
ATROPHY, FOCAL					1 (7%)
#STOMACH	(18)	(39)	(29)	(32)	(14)
HYPERPLASIA, PLASMA CELL				1 (3%)	
#LARGE INTESTINE	(13)	(36)	(28)	(32)	(11)
NEMATODIASIS			1 (4%)		
URINARY SYSTEM					
#KIDNEY	(20)	(39)	(33)	(33)	(13)
INFLAMMATION, INTERSTITIAL			1 (3%)		
INFLAMMATION, SUPPURATIVE			3 (9%)		
PYELONEPHRITIS SUPPURATIVE				1 (3%)	
INFLAMMATION, ACUTE SUPPURATIVE			1 (3%)		
INFLAMMATION, CHRONIC				2 (6%)	1 (8%)
INFLAMMATION, CHRONIC FOCAL		1 (3%)	1 (3%)		
INFLAMMATION, CHRONIC DIFFUSE		1 (3%)			
NECROSIS, COAGULATIVE			1 (3%)		
NECROSIS, MEDULLARY		3 (8%)	6 (18%)		
#KIDNEY/CORTEX	(20)	(39)	(33)	(33)	(13)
INFLAMMATION, SUPPURATIVE			1 (3%)		
#KIDNEY/MEDULLA	(20)	(39)	(33)	(33)	(13)
ABSCESS, NOS				1 (3%)	
NECROSIS, COAGULATIVE				1 (3%)	
#KIDNEY/TUBULE	(20)	(39)	(33)	(33)	(13)
CAST, NOS			2 (6%)		
CALCIFICATION, NOS			1 (3%)		
#KIDNEY/PELVIS	(20)	(39)	(33)	(33)	(13)
ABSCESS, NOS				1 (3%)	1 (8%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D4. CONTROL AND TREATED FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	LOW DOSE VEHICLE CONTROL	LOW DOSE	LOWER MID DOSE	UPPER MID DOSE	HIGH DOSE
ENDOCRINE SYSTEM					
NONE					
REPRODUCTIVE SYSTEM					
#UTERUS	(20)	(39)	(31)	(32)	(15)
CYST, NOS			2 (6%)		
PYOMETRA			2 (6%)		
#UTERUS/ENDOMETRIUM	(20)	(39)	(31)	(32)	(15)
HYPERPLASIA, CYSTIC	2 (10%)	21 (54%)	7 (23%)		
#OVARY/OVIDUCT	(20)	(39)	(31)	(32)	(15)
INFLAMMATION, SUPPURATIVE				1 (3%)	
#OVARY	(20)	(39)	(27)	(29)	(11)
CYST, NOS			1 (4%)		
FOLLICULAR CYST, NOS		1 (3%)			
INFLAMMATION, SUPPURATIVE			1 (4%)	1 (3%)	
ATROPHY, NOS				1 (3%)	
NERVOUS SYSTEM					
NONE					
SPECIAL SENSE ORGANS					
NONE					
MUSCULOSKELETAL SYSTEM					
*SKELETAL MUSCLE	(20)	(39)	(33)	(33)	(32)
INFLAMMATION, FOCAL			1 (3%)		
BODY CAVITIES					
*ABDOMINAL WALL	(20)	(39)	(33)	(33)	(32)
ABSCESS, NOS					1 (3%)
*PERITONEUM	(20)	(39)	(33)	(33)	(32)
INFLAMMATION, NOS			2 (6%)		

# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D4. CONTROL AND TREATED FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	LOW DOSE VEHICLE CONTROL	LOW DOSE	LOWER MID DOSE	UPPER MID DOSE	HIGH DOSE
INFLAMMATION, SUPPURATIVE		2 (5%)	8 (24%)	1 (3%)	
INFLAMMATION, FIBRINOUS				4 (12%)	
ABSCESS, NOS				1 (3%)	
INFLAMMATION, ACUTE/CHRONIC			3 (9%)		
INFLAMMATION, CHRONIC		1 (3%)	9 (27%)	7 (21%)	2 (6%)
INFLAMMATION, CHRONIC FOCAL			1 (3%)		
INFLAMMATION, CHRONIC SUPPURATIV			1 (3%)	1 (3%)	
FIBROSIS				1 (3%)	
*PLEURA	(20)	(39)	(33)	(33)	(32)
INFLAMMATION, NOS				1 (3%)	
INFLAMMATION, SUPPURATIVE			1 (3%)		
INFLAMMATION, FIBRINOUS				1 (3%)	
ALL OTHER SYSTEMS					
*MULTIPLE ORGANS	(20)	(39)	(33)	(33)	(32)
HYPERPLASIA, LYMPHOID		1 (3%)			
SPECIAL MORPHOLOGY SUMMARY					
NO LESION REPORTED		4	1	8	9
NECROPSY PERF/NO HISTO PERFORMED					17
AUTOLYSIS/NO NECROPSY		1	2	2	3

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

NIH LIBRARY

APPENDIX E

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS IN RATS GIVEN INTRAPERITONEAL INJECTIONS OF ACRONYCINE

NIH LIBRARY

Table E1. Time-adjusted Analyses of the Incidence of Primary Tumors in Male Rats Given Intraperitoneal Injections of Acronycine[a], Using Vehicle Controls

Topography: Morphology	Low-Dose Vehicle Control	Mid- and High-Dose Vehicle Control	Low Dose	Mid Dose	High Dose
Liver: Hepatocellular Adenoma or Carcinoma[b] (52)	0/10 (0)	0/8 (0)	2/21 (10)	0/11 (0)	0/0 (-)
P Values[c,d]	--	--	N.S.	--	--
Relative Risk (Vehicle Control)[f]			Infinite	--	--
Lower Limit			0.156	--	--
Upper Limit			Infinite	--	--
Weeks to First Observed Tumor	--	--	80	--	--
Liver: Osteosarcoma[b] (41)	0/10 (0)	0/8 (0)	0/25 (0)	0/18 (0)	2/7 (29)
P Values[c,d]	--	P = 0.048	--	--	N.S.
Relative Risk (Vehicle Control)[f]			--	--	Infinite
Lower Limit			--	--	0.392
Upper Limit			--	--	Infinite
Weeks to First Observed Tumor	--	--	--	--	41

Table E1. Time-adjusted Analyses of the Incidence of Primary Tumors in Male Rats Given Intraperitoneal Injections of Acronycine[a], Using Vehicle Controls

(continued)

Topography: Morphology	Low-Dose Vehicle Control	Mid- and High-Dose Vehicle Control	Low Dose	Mid Dose	High Dose
Musculoskeletal System:					
Osteosarcoma[b] (33)	0/10 (0)	0/8 (0)	3/29 (10)	11/26 (42)	8/15 (53)
P Values[c,d]	--	P = 0.019	N.S.	P = 0.027	P = 0.013
Relative Risk (Vehicle Control)[f]			Infinite	Infinite	Infinite
Lower Limit			0.231	1.192	1.442
Upper Limit			Infinite	Infinite	Infinite
Weeks to First Observed Tumor	--	--	50	35	33
All Sites:					
Hemangiosarcoma[b] (46)	0/10 (0)	0/8 (0)	4/23 (17)	0/13 (0)	1/4 (25)
P Values[c,d]	--	N.S.	N.S.	--	N.S.
Relative Risk (Vehicle Control)[f]			Infinite	--	Infinite
Lower Limit			0.452	--	0.117
Upper Limit			Infinite	--	Infinite
Weeks to First Observed Tumor	--	--	48	--	46

Table E1. Time-adjusted Analyses of the Incidence of Primary Tumors in Male Rats Given Intraperitoneal Injections of Acronycine[a], Using Vehicle Controls

(continued)

Topography: Morphology	Low-Dose Vehicle Control	Mid- and High-Dose Vehicle Control	Low Dose	Mid Dose	High Dose
All Sites· Osteosarcoma[b] (25)	0/10 (0)	0/8 (0)	4/31 (13)	13/30 (43)	12/18 (67)
P Values[c,d]	--	P = 0.002	N.S.	P = 0.022	P = 0.002
Relative Risk (Vehicle Control)[f]			Infinite	Infinite	Infinite
Lower Limit			0.334	1.248	1.941
Upper Limit			Infinite	Infinite	Infinite
Weeks to First Observed Tumor	--	--	50	35	25
Bile Duct: Bile Duct Carcinoma[b] (52)	0/10 (0)	0/8 (0)	2/20 (10)	0/11 (0)	0/0 (-)
P Values[c,d]	--	--	N.S.	--	--
Relative Risk (Vehicle Control)[f]			Infinite	--	--
Lower Limit			0.164	--	--
Upper Limit			Infinite	--	--
Weeks to First Observed Tumor	--	--	62	--	--

Table E1. Time-adjusted Analyses of the Incidence of Primary Tumors in Male Rats Given Intraperitoneal Injections of Acronycine[a], Using Vehicle Controls

(continued)

Topography: Morphology	Low-Dose Vehicle Control	Mid- and High-Dose Vehicle Control	Low Dose	Mid Dose	High Dose
Adrenal: Cortical Adenoma[b] (41)	0/10 (0)	1/8 (13)	1/24 (4)	2/18 (11)	4/7 (57)
P Values[c,d]	--	P = 0.045	N.S.	N.S.	N.S.
Relative Risk (Vehicle Control)[f]			Infinite	0.889	4.571
Lower Limit			0.024	0.058	0.629
Upper Limit			Infinite	49.343	153.053
Weeks to First Observed Tumor	--	72	80	61	41
Adrenal: Cortical Adenoma or Carcinoma[b] (41)	0/10 (0)	1/8 (13)	1/24 (4)	3/18 (17)	4/7 (57)
P Values[c,d]	--	N.S.	N.S.	N.S.	N.S.
Relative Risk (Vehicle Control)[f]			Infinite	1.333	4.571
Lower Limit			0.024	0.138	0.629
Upper Limit			Infinite	65.560	153.053
Weeks to First Observed Tumor	--	72	80	61	41

Table E1. Time-adjusted Analyses of the Incidence of Primary Tumors in Male Rats Given Intraperitoneal Injections of Acronycine[a], Using Vehicle Controls

(continued)

Topography: Morphology	Low-Dose Vehicle Control	Mid- and High-Dose Vehicle Control	Low Dose	Mid Dose	High Dose
Peritoneum: Sarcoma, NOS[b] (32)	0/10 (0)	0/8 (0)	2/29 (7)	0/26 (0)	1/16 (6)
P Values[c,d]	--	N.S.	N.S.	--	N.S.
Relative Risk (Vehicle Control)[f]			Infinite	--	Infinite
Lower Limit			0.112	--	0.030
Upper Limit			Infinite	--	Infinite
Weeks to First Observed Tumor	--	--	56	--	32
Peritoneum: Fibrosarcoma[b] (32)	0/10 (0)	0/8 (0)	0/29 (0)	0/26 (0)	2/16 (13)
P Values[c,d]	--	N.S.	--	--	N.S.
Relative Risk (Vehicle Control)[f]			--	--	Infinite
Lower Limit			--	--	0.169
Upper Limit			--	--	Infinite
Weeks to First Observed Tumor	--	--	--	--	32

Table E1. Time-adjusted Analyses of the Incidence of Primary Tumors in Male Rats Given Intraperitoneal Injections of Acronycine[a], Using Vehicle Controls

(continued)

Topography: Morphology	Low-Dose Vehicle Control	Mid- and High-Dose Vehicle Control	Low Dose	Mid Dose	High Dose
Peritoneum: Mesothelioma[b] (52)	0/10 (0)	0/8 (0)	3/20 (15)	1/11 (9)	0/0 (-)
P Values[c,d]	--	N.S.	N.S.	N.S.	--
Relative Risk (Vehicle Control)[f]			Infinite	Infinite	--
Lower Limit			0.336	0.044	--
Upper Limit			Infinite	Infinite	--
Weeks to First Observed Tumor	--	--	58	55	--
Peritoneum: Sarcoma and Other Related Tumors[b] (32)[+]	0/10 (0)	0/8 (0)	5/30 (17)	3/26 (12)	7/16 (44)
P Values[c,d]	--	P = 0.006	N.S.	N.S.	P = 0.033
Relative Risk (Vehicle Control)[f]			Infinite	Infinite	Infinite
Lower Limit			0.470	0.213	1.142
Upper Limit			Infinite	Infinite	Infinite
Weeks to First Observed Tumor	--	--	56	55	32

[+] These tumors consist of sarcoma, fibrosarcoma, or mesothelioma.

Table E1. Time-adjusted Analyses of the Incidence of Primary Tumors in Male Rats Given Intraperitoneal Injections of Acronycine[a], Using Vehicle Controls

(continued)

Topography: Morphology	Low-Dose Vehicle Control	Mid- and High-Dose Vehicle Control	Low Dose	Mid Dose	High Dose
Multiple Organs:					
Fibrosarcoma[b] (33)	0/10 (0)	0/8 (0)	0/29 (0)	2/26 (8)	2/15 (13)
P Values[c,d]	--	N.S.	--	N.S.	N.S.
Relative Risk (Vehicle Control)[f]			--	Infinite	Infinite
Lower Limit			--	0.104	0.181
Upper Limit			--	Infinite	Infinite
Weeks to First Observed Tumor	--	--	--	61	33

[a] Treated groups received doses of 3.75, 7.5, or 15 mg/kg by intraperitoneal injection.

[b] Number of tumor-bearing animals/number of animals examined at site (percent), based on animals that survived at least as long as the number of weeks on study shown in parentheses after the description of morphology.

[c] Beneath the incidence of tumors in the mid- and high-dose control group is the probability level for the Cochran-Armitage test when $P < 0.05$ using only the mid- and high-dose groups in the trend analysis; otherwise, not significant (N.S.) is indicated. Beneath the incidence of tumors in a treated group is the probability level for the Fisher exact test for the comparison of that treated group with its appropriate control group when $P < 0.05$; otherwise, not significant (N.S.) is indicated.

Table E1. Time-adjusted Analyses of the Incidence of Primary Tumors in Male Rats Given Intraperitoneal Injections of Acronycine[a], Using Vehicle Controls

(continued)

---

[d] A negative trend (N) indicates a lower incidence in a treated group than in a control group.

[e] The probability level for departure from linear trend is given when $P < 0.05$ for any comparison.

[f] The 95% confidence interval of the relative risk between each treated group and its appropriate control group.

Table E2. Time-adjusted Analyses of the Incidence of Primary Tumors in Female Rats Given Intraperitoneal Injections of Acronycine[a], Using Vehicle Controls

Topography: Morphology	Low-Dose Vehicle Control	Mid- and High-Dose Vehicle Control	Low Dose	Mid Dose	High Dose
Subcutaneous Tissue: Fibroma[b] (52)	0/10 (0)	0/9 (0)	2/34 (6)	0/29 (0)	1/14 (7)
P Values[c,d]	--	N.S.	N.S.	--	N.S.
Relative Risk (Vehicle Control)[f]			Infinite	--	Infinite
Lower Limit			0.096	--	0.037
Upper Limit			Infinite	--	Infinite
Weeks to First Observed Tumor	--	--	79	--	80
Subcutaneous Tissue: Fibrosarcoma[b] (52)	0/10 (0)	0/9 (0)	1/34 (3)	2/29 (7)	0/14 (0)
P Values[c,d]	--	N.S.	N.S.	N.S.	--
Relative Risk (Vehicle Control)[f]			Infinite	Infinite	--
Lower Limit			0.017	0.103	--
Upper Limit			Infinite	Infinite	--
Weeks to First Observed Tumor	--	--	58	56	--

NIH LIBRARY

Table E2. Time-adjusted Analyses of the Incidence of Primary Tumors in Female Rats Given Intraperitoneal Injections of Acronycine[a], Using Vehicle Controls

(continued)

Topography: Morphology	Low-Dose Vehicle Control	Mid- and High-Dose Vehicle Control	Low Dose	Mid Dose	High Dose
Subcutaneous Tissue: Fibroma or Fibrosarcoma[b] (52)	0/10 (0)	0/9 (0)	3/34 (9)	2/29 (7)	1/14 (7)
P Values[c,d]	--	N.S.	N.S.	N.S.	N.S.
Relative Risk (Vehicle Control)[f]			Infinite	Infinite	Infinite
Lower Limit			0.197	0.103	0.037
Upper Limit			Infinite	Infinite	Infinite
Weeks to First Observed Tumor	--	--	58	56	80
Lung: Alveolar/Bronchiolar Adenoma[b] (32)	0/10 (0)	0/8 (0)	0/35 (0)	2/31 (6)	0/28 (0)
P Values[c,d]	--	N.S.	--	N.S.	N.S.
Relative Risk (Vehicle Control)[f]			--	Infinite	--
Lower Limit			--	0.087	--
Upper Limit			--	Infinite	--
Weeks to First Observed Tumor	--	--	--	32	--

Table E2. Time-adjusted Analyses of the Incidence of Primary Tumors in Female Rats Given Intraperitoneal Injections of Acronycine[a], Using Vehicle Controls

(continued)

Topography: Morphology	Low-Dose Vehicle Control	Mid- and High-Dose Vehicle Control	Low Dose	Mid Dose	High Dose
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma[b] (32)	0/10 (0)	1/8 (13)	1/35 (3)	2/31 (6)	0/28 (0)
P Values[c,d]	--	N.S.	N.S.	N.S.	N.S.
Relative Risk (Vehicle Control)[f]			Infinite	0.516	0.000
Lower Limit			0.017	0.034	0.000
Upper Limit			Infinite	29.485	5.278
Weeks to First Observed Tumor	--	82	76	32	--
Liver: Hepatocellular Adenoma or Carcinoma[b] (52)	0/9 (0)	0/8 (0)	0/34 (0)	4/29 (14)	0/13 (0)
P Values[c,d]	--	N.S.	--	N.S.	--
Relative Risk (Vehicle Control)[f]			--	Infinite	--
Lower Limit			--	0.296	--
Upper Limit			--	Infinite	--
Weeks to First Observed Tumor	--	--	--	64	--

Table E2. Time-adjusted Analyses of the Incidence of Primary Tumors in Female Rats Given Intraperitoneal Injections of Acronycine[a], Using Vehicle Controls

(continued)

Topography: Morphology	Low-Dose Vehicle Control	Mid- and High-Dose Vehicle Control	Low Dose	Mid Dose	High Dose
Adrenal: Cortical Adenoma or Carcinoma[b] (40)	0/10 (0)	1/8 (13)	0/32 (0)	9/28 (32)	7/23 (30)[+]
P Values[c,d]	--	N.S.	--	N.S.	N.S.
Relative Risk (Vehicle Control)[f]			--	2.571	2.435
Lower Limit			--	0.477	0.418
Upper Limit			--	107.105	103.211
Weeks to First Observed Tumor	--	82	--	58	40
Mammary Gland: Adenocarcinoma, NOS[b] (32)	0/10 (0)	0/8 (0)	6/35 (17)	4/34 (12)	2/30 (7)
P Values[c,d]	--	N.S.	N.S.	N.S.	N.S.
Relative Risk (Vehicle Contol)[f]			Infinite	Infinite	Infinite
Lower Limit			0.512	0.252	0.090
Upper Limit			Infinite	Infinite	Infinite
Weeks to First Observed Tumor	--	--	73	32	61

[+] One animal in this group was diagnosed with both adenoma and carcinoma.

Table E2. Time-adjusted Analyses of the Incidence of Primary Tumors in Female Rats Given Intraperitoneal Injections of Acronycine[a], Using Vehicle Controls

(continued)

Topography: Morphology	Low-Dose Vehicle Control	Mid- and High-Dose Vehicle Control	Low Dose	Mid Dose	High Dose
Mammary Gland: Fibroma[b] (52)	0/10 (0)	0/8 (0)	0/34 (0)	2/29 (7)	1/14 (7)
P Values[c,d]	--	N.S.	--	N.S.	N.S.
Relative Risk (Vehicle Control)[f]			--	Infinite	Infinite
Lower Limit			--	0.093	0.034
Upper Limit			--	Infinite	Infinite
Weeks to First Observed Tumor	--	--	--	82	80
Mammary Gland: Fibroadenoma[b] (51)	1/10 (10)	3/8 (38)	20/34 (59)	13/29 (45)	3/15 (20)
P Values[c,d]	--	N.S.	P = 0.007	N.S.	N.S.
Relative Risk (Vehicle Control)[f]			5.882	1.195	0.533
Lower Limit			1.209	0.491	0.104
Upper Limit			227.093	5.422	3.235
Weeks to First Observed Tumor	80	82	76	51	61

Table E2. Time-adjusted Analyses of the Incidence of Primary Tumors in Female Rats Given Intraperitoneal Injections of Acronycine[a], Using Vehicle Controls

(continued)

Topography: Morphology	Low-Dose Vehicle Control	Mid- and High-Dose Vehicle Control	Low Dose	Mid Dose	High Dose
Mammary Gland: All Tumors Except Fibroma[b] (32)+	1/10 (10)	3/8 (38)	22/35 (63)	16/34 (47)	5/30 (17)
P Values[c,d]	--	P = 0.034(N)	P = 0.004	N.S.	N.S.
Relative Risk (Vehicle Control)[f]			6.286	1.255	0.444
Lower Limit			1.310	0.535	0.128
Upper Limit			237.797	5.627	2.471
Weeks to First Observed Tumor	80	82	66	32	61
Uterus: Endometrial Stromal Polyp[b] (45)	0/10 (0)	0/8 (0)	5/34 (15)	0/30 (0)	1/19 (5)
P Values[c,d]	--	N.S.	N.S.	--	N.S.
Relative Risk (Vehicle Control)[f]			Infinite	--	Infinite
Lower Limit			0.415	--	0.025
Upper Limit			Infinite	--	Infinite
Weeks to First Observed Tumor	--	--	80	--	45

+These tumors consist of adenocarcinoma, fibroadenoma, papillary adenocarcinoma, cystadenoma, or cystadenocarcinoma. The fibromas are omitted from this combination.

Table E2. Time-adjusted Analyses of the Incidence of Primary Tumors in Female Rats Given Intraperitoneal Injections of Acronycine[a], Using Vehicle Controls

(continued)

Topography: Morphology	Low-Dose Vehicle Control	Mid- and High-Dose Vehicle Control	Low Dose	Mid Dose	High Dose
Peritoneum: Sarcoma, NOS[b] (42)	0/10 (0)	0/8 (0)	0/35 (0)	0/30 (0)	5/22 (23)
P Values[c,d]	--	P = 0.010	--	--	N.S.
Relative Risk (Vehicle Control)[f]			--	--	Infinite
Lower Limit			--	--	0.533
Upper Limit			--	--	Infinite
Weeks to First Observed Tumor	--	--	--	--	42
Peritoneum: Fibrosarcoma[b] (52)	0/10 (0)	0/8 (0)	0/34 (0)	2/29 (7)	0/14 (0)
P Values[c,d]	--	N.S.	--	N.S.	--
Relative Risk (Vehicle Control)[f]			--	Infinite	--
Lower Limit			--	0.093	--
Upper Limit			--	Infinite	--
Weeks to First Observed Tumor	--	--	--	58	--

Table E2. Time-adjusted Analyses of the Incidence of Primary Tumors in Female Rats Given Intraperitoneal Injections of Acronycine[a], Using Vehicle Controls

(continued)

Topography: Morphology	Low-Dose Vehicle Control	Mid- and High-Dose Vehicle Control	Low Dose	Mid Dose	High Dose
Peritoneum: Mesothelioma[b] (43)	0/10 (0)	0/8 (0)	1/35 (3)	0/30 (0)	2/21 (10)
P Values[c,d]	--	N.S.	N.S.	--	N.S.
Relative Risk (Vehicle Control)[f]			Infinite	--	Infinite
Lower Limit			0.017	--	0.129
Upper Limit			Infinite	--	Infinite
Weeks to First Observed Tumor	--	--	46	--	43
Peritoneum: Sarcoma and Other Related Tumors[b] (34)[+]	0/10 (0)	0/8 (0)	1/35 (3)	5/30 (17)	13/28 (46)
P Values[c,d]	--	P = 0.002	N.S.	N.S.	P = 0.016
Relative Risk (Matched Control)[f]			Infinite	Infinite	Infinite
Lower Limit			0.017	0.390	1.339
Upper Limit			Infinite	Infinite	Infinite
Weeks to First Observed Tumor	--	--	46	51	34

[+] These tumors consist of sarcoma, fibrosarcoma, or mesothelioma.

Table E2. Time-adjusted Analyses of the Incidence of Primary Tumors in Female Rats Given Intraperitoneal Injections of Acronycine[a], Using Vehicle Controls

(continued)

Topography: Morphology	Low-Dose Vehicle Control	Mid- and High-Dose Vehicle Control	Low Dose	Mid Dose	High Dose
Multiple Organs: Sarcoma, NOS[b] (34)	0/10 (0)	0/8 (0)	0/35 (0)	2/30 (7)	3/29 (10)
P Values[c,d]	--	N.S.	--	N.S.	N.S.
Relative Risk (Vehicle Control)[f]			--	Infinite	Infinite
Lower Limit			--	0.090	0.192
Upper Limit			--	Infinite	Infinite
Weeks to First Observed Tumor	--	--	--	51	34
Multiple Organs: Fibrosarcoma[b] (41)	0/10 (0)	0/8 (0)	0/35 (0)	2/30 (7)	1/25 (4)
P Values[c,d]	--	N.S.	--	N.S.	N.S.
Relative Risk (Vehicle Control)[f]			--	Infinite	Infinite
Lower Limit			--	0.090	0.019
Upper Limit			--	Infinite	Infinite
Weeks to First Observed Tumor	--	--	--	51	41

Table E2. Time-adjusted Analyses of the Incidence of Primary Tumors in Female Rats Given Intraperitoneal Injections of Acronycine[a], Using Vehicle Controls

(continued)

Topography: Morphology	Low-Dose Vehicle Control	Mid- and High-Dose Vehicle Control	Low Dose	Mid Dose	High Dose
All Sites: Osteosarcoma[b] (52)	0/10 (0)	0/8 (0)	0/32 (0)	0/22 (0)	1/8 (13)
P Values[c,d]	--	N.S.	--	--	N.S.
Relative Risk (Matched Control)[f]			--	--	Infinite
Lower Limit			--	--	0.059
Upper Limit			--	--	Infinite
Weeks to First Observed Tumor	--	--	--	--	65
All Sites: Hemangiosarcoma[b] (52)	0/10 (0)	0/8 (0)	5/34 (15)	0/29 (0)	1/14 (7)
P Values[c,d]	--	N.S.	N.S.	--	N.S.
Relative Risk (Vehicle Control)[f]			Infinite	--	Infinite
Lower Limit			0.415	--	0.034
Upper Limit			Infinite	--	Infinite
Weeks to First Observed Tumor	--	--	70	--	80

Table E2. Time-adjusted analyses of the Incidence of Primary Tumors in Female Rats Given Intraperitoneal Injections of Acronycine[a], Using Vehicle Controls

(continued)

---

[a] Treated groups received doses of 3.75, 7.5, or 15 mg/kg by intraperitoneal injection.

[b] Number of tumor-bearing animals/number of animals examined at site (percent), based on animals that survived at least as long as the number of weeks on study shown in parentheses after the description of morphology.

[c] Beneath the incidence of tumors in the mid- and high-dose control group is the probability level for the Cochran-Armitage test when $P < 0.05$ using only the mid- and high-dose groups in the trend analysis; otherwise, not significant (N.S.) is indicated. Beneath the incidence of tumors in a treated group is the probability level for the Fisher exact test for the comparison of that treated group with its appropriate control group when $P < 0.05$; otherwise, not significant (N.S.) is indicated.

[d] A negative trend (N) indicates a lower incidence in a treated group than in a control group.

[e] The probability level for departure from linear trend is given when $P < 0.05$ for any comparison.

[f] The 95% confidence interval of the relative risk between each treated group and its appropriate control group.

NIH Library

APPENDIX F

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS IN MICE GIVEN INTRAPERITONEAL INJECTIONS OF ACRONYCINE

NIH LIBRARY

Table F1. Time-adjusted Analyses of the Incidence of Primary Tumors in Male Mice Given Intraperitoneal Injections of Acronycine[a], Using Vehicle Controls

Topography: Morphology	Low-Dose Vehicle Control	Low Dose
Hematopoietic System: Lymphoma[b] (33)	13/17 (76)	10/37 (27)
P Values[c,d]		$P < 0.001$(N)
Relative Risk (Vehicle Control)[e]		0.353
Lower Limit		0.218
Upper Limit		0.687
Weeks to First Observed Tumor	33	89
Multiple Organs: Sarcoma, NOS[b] (52)	0/5 (0)	2/33 (6)
P Values[c,d]		N.S.
Relative Risk (Vehicle Control)[e]		Infinite
Lower Limit		0.057
Upper Limit		Infinite
Weeks to First Observed Tumor	--	81

Table F1. Time-adjusted Analyses of the Incidence of Primary Tumors in Male Mice Given Intraperitoneal Injections of Acronycine[a], Using Vehicle Controls

(continued)

---

[a] The low-dose group received 2 mg/kg.

[b] Number of tumor-bearing animals/number of animals examined at site (percent), based on animals that survived at least as long as the number of weeks on study shown in parentheses after the description of morphology.

[c] Beneath the incidence of tumors in a treated group is the probability level for the Fisher exact test for the comparison of the treated group with the vehicle-control group when $P < 0.05$; otherwise, not significant (N.S.) is indicated.

[d] A negative trend (N) indicates a lower incidence in a treated group than in a control group.

[e] The 95% confidence interval of the relative risk between the treated group and the control group.

Table F2. Time-adjusted Analyses of the Incidence of Primary Tumors in Female Mice Given Intraperitoneal Injections of Acronycine[a], Using Vehicle Controls

Topography: Morphology	Low-Dose Vehicle Control	Low Dose
Hematopoietic System: Lymphoma[b] (28)	19/19 (100)	6/37 (16)
P Values[c,d]		$P < 0.001$ (N)
Relative Risk (Vehicle Control)[e]		0.162
Lower Limit		0.000
Upper Limit		0.289
Weeks to First Observed Tumor	28	74
Multiple Organs: Sarcoma, NOS[b] (52)	0/2 (0)	2/31 (6)
P Values[c,d]		N.S.
Relative Risk (Vehicle Control)[e]		Infinite
Lower Limit		0.039
Upper Limit		Infinite
Weeks to First Observed Tumor	--	85

Table F2. Time-adjusted Analyses of the Incidence of Primary Tumors in Female Mice Given Intraperitoneal Injections of Acronycine[a], Using Vehicle Controls

(continued)

---

[a] The low-dose group received 2 mg/kg.

[b] Number of tumor-bearing animals/number of animals examined at site (percent), based on animals that survived at least as long as the number of weeks on study shown in parentheses after the description of morphology.

[c] Beneath the incidence of tumors in a treated group is the probability level for the Fisher exact test for the comparison of the treated group with the vehicle-control group when $P < 0.05$; otherwise, not significant (N.S.) is indicated.

[d] A negative trend (N) indicates a lower incidence in a treated group than in a control group.

[e] The 95% confidence interval of the relative risk between the treated group and the control group.

Review of the Bioassay of Acronycine* for Carcinogenicity by the Data Evaluation/Risk Assessment Subgroup of the Clearinghouse on Environmental Carcinogens

March 7, 1978

The Clearinghouse on Environmental Carcinogens was established in May, 1976, in compliance with DHEW Committee Regulations and the Provisions of the Federal Advisory Committee Act. The purpose of the Clearinghouse is to advise the Director of the National Cancer Institute (NCI) on its bioassay program to identify and to evaluate chemical carcinogens in the environment to which humans may be exposed. The members of the Clearinghouse have been drawn from academia, industry, organized labor, public interest groups, State health officials, and quasi-public health and research organizations. Members have been selected on the basis of their experience in carcinogenesis or related fields and, collectively, provide expertise in chemistry, biochemistry, biostatistics, toxicology, pathology, and epidemiology. Representatives of various Governmental agencies participate as ad hoc members. The Data Evaluation/Risk Assessment Subgroup of the Clearinghouse is charged with the responsibility of providing a peer review of reports prepared on NCI-sponsored bioassays of chemicals studied for carcinogenicity. It is in this context that the below critique is given on the bioassay of Acronycine for carcinogenicity.

The primary reviewer for the report on the bioassay of Acronycine described the experimental design and conditions under which Acronycine was tested. A dose-related incidence of osteosarcomas occurred in the high dose male rats; one osteosarcoma was observed among the treated females. Other tumors were reported in the peritoneal cavity in both sexes of treated rats. A statistically significant increase also was found in the incidence of mammary gland tumors in treated female rats. Although survival was inadequate to evaluate the carcinogenicity of Acronycine in mice, increases in lymphomas were observed among the treated animals. The primary criticism of the study was the use of excessively high dose levels. Since Acronycin is used as a chemotherapeutic agent, the primary reviewer said that it should receive special consideration in assessing human risk.

The secondary reviewer commented that Acronycine was probably carcinogenic, although he said the study was deficient. Another Subgroup member agreed with the conclusion given in the report. However, he felt that the value of the study was diminished as a result of the excessively high dose levels administered and the fact that animals were housed in the same room in which other chemicals were under study.

A motion was made that the report on the bioassay of Acronycine be accepted as written. The motion was seconded and approved unanimously.

Members present were:

Gerald N. Wogan (Chairman), Massachusetts Institute of Technology
Arnold Brown, Mayo Clinic
E. Cuyler Hammond, American Cancer Society
Joseph Highland, Environmental Defense Fund
Henry Pitot, McArdle Laboratory
George Roush, Jr., Monsanto Company
Michael Shimkin, University of California at San Diego

---

* Subsequent to this review, changes may have been made in the bioassay report either as a result of the review or other reasons. Thus, certain comments and criticisms reflected in the review may no longer be appropriate.

NIH LIBRARY

☆U.S. GOVERNMENT PRINTING OFFICE: 1978-260-899/3087

Library
National Institutes of Health
Bethesda, Maryland 20014

NIH LIBRARY
Amazing Research.
Amazing Help.
http://nihlibrary.nih.gov

10 Center Drive
Bethesda, MD 20892-1150
301-496-1080

DATE DUE

GAYLORD
PRINTED IN U.S.A.

NIH LIBRARY
4 0128 2713

NIH LIBRARY
3 1496 00185 2758

DHEW Publication No. (NIH) 78-849